EXHIBIT K

PI: **BenMohamed, Lbachir**	Title: PROTECTIVE IMMUNITY AGAINST RECURRENT OCULAR HERPES INDUCED WITH SELF-ASSEMBLING PROTEIN NANOPARTICLES	
Received: 12/10/2018	FOA: PA18-489 Clinical Trial:Not Allowed	Council: 05/2019
Competition ID: FORMS-E	FOA Title: NIH Exploratory/Developmental Research Grant Program (Parent R21 Clinical Trial Not Allowed)	
1 R21 AI147499-01	Dual:	Accession Number: 4247300
IPF: 577504	Organization: UNIVERSITY OF CALIFORNIA-IRVINE	
Former Number:	Department: Ophthalmology Research	
IRG/SRG: ZRG1 BDCN-J (81)S	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 125,000 Year 2: 150,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
LBACHIR BENMOHAMED	The Regents of the University of California, Irvine	PD/PI
CHRISTINE MCLAREN	The Regents of the University of California, Irvine	Other (Specify)-Collaborator
Mohammed Bouziane	Sunomix Therapeutics Inc	Co-Investigator
PETER BURKHARD	Alpha-O Peptides AG	Other (Specify)-Collaborator

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2018-12-10

Application Identifier

5. APPLICANT INFORMATION **Organizational DUNS*:** 046705849

Legal Name*: The Regents of the University of California, Irvine
Department:
Division:
Street1*: 141 Innovation Drive, Suite 250
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600

Person to be contacted on matters involving this application
Prefix: First Name*: Jasmin Middle Name: Last Name*: Ramirez Suffix:
Position/Title: CONTRACT & GRANT OFFICER
Street1*: 141 Innovation, Suite 250
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600
Phone Number*: 9498242460 Fax Number: 9498242094 Email: jasminjr@uci.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-952226406-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
PROTECTIVE IMMUNITY AGAINST RECURRENT OCULAR HERPES INDUCED WITH SELF-ASSEMBLING PROTEIN NANOPARTICLES

12. PROPOSED PROJECT
Start Date* 07/01/2019
Ending Date* 06/30/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-045

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: LBACHIR Middle Name: Last Name*: BENMOHAMED Suffix:
Position/Title: Professor/Director
Organization Name*: The Regents of the University of California, Irvine
Department: Ophthalmology Research
Division: School of Medicine
Street1*: Hewitt Hall Room 2032
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600
Phone Number*: (949) 824-8937 Fax Number: (949) 824-9626 Email*: lbenmoha@uci.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$402,063.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$402,063.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Jasmin Middle Name: Last Name*: Ramirez Suffix:
Position/Title*: CONTRACT & GRANT OFFICER
Organization Name*: The Regents of the University of California, Irvine
Department:
Division:
Street1*: 141 Innovation, Suite 250
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92697-7600
Phone Number*: 9498242460 Fax Number: 9498242094 Email*: jasminjr@uci.edu

Signature of Authorized Representative*
Jasmin Ramirez

Date Signed*
12/10/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:CoverLetterEye1010649549.pdf

424 R&R and PHS-398 Specific
Table Of Contents

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Regents of the University of California Irvine
Duns Number: 046705849
Street1*: Hewitt Hall Room 2032
Street2: Suite 150
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92697-4390
Project/Performance Site Congressional District*: CA-045

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Sunomix Therapeutics Inc
DUNS Number: 080437688
Street1*: 3210 Merryfield Row, , CA 92121
Street2:
City*: San Diego
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92121-1126
Project/Performance Site Congressional District*: CA-049

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

6255

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract1010649535.pdf
8. Project Narrative*	ProjectNarrative1010649548.pdf
9. Bibliography & References Cited	LiteratureCited1010649536.pdf
10.Facilities & Other Resources	FacilitiesUCISunomix1010649537.pdf
11.Equipment	Equipment_1010649550.pdf

Funding Opportunity Number: PA-18-489. Received Date: 2018-12-10T19:07:05.000-05:00

PROJECT SUMMARY/ABSTRACT

A staggering number of individuals—over 3 billion worldwide—are currently infected with herpes simplex virus type 1 (HSV-1), which causes frequent, and often lifelong, bouts of recurrent corneal herpetic disease. This potentially blinding disease is a result of corneal re-infection following reactivation of latent HSV-1 from sensory neurons of the trigeminal ganglia (TG). Our long-term goal is to develop a vaccine to protect against ocular herpes infection and disease. The most recent herpes vaccine clinical trials that used a recombinant HSV glycoprotein D (gD)-based subunit antigen vaccine mixed with monophosphoryl lipid A (MPLA) adjuvant and delivered intramuscularly failed to protect despite inducing strong HSV-specific neutralizing antibodies. Results from those clinical trials emphasize two major gaps in knowledge: (1) The need to design an alternative antigen delivery system that will induce cell-mediated immune responses (in addition to humoral responses). (2) The need to design an ocular herpes vaccine that will include T cell-induced HSV antigens (Ags), other than the gD. HSV-specific TG-resident $CD8^+$ T cells play a critical role in aborting reactivation of HSV-1 from latently infected sensory neurons, and the involvement of ocular mucosal surface- (OMS-) resident $CD4^+$ T cells is gaining wider acceptance. Our recently published and preliminary data demonstrate that: (A) $CD8^+$ T cells from "naturally protected" HSV-seropositive ASYMP individuals mainly recognize T cell epitopes from HSV tegument proteins VP16 and VP22. (B) Immunization of B6 6255 with Self Assembling Protein Nanoparticles (SAPNs) that incorporate an HSV-1 CD8+ T cell epitope together with a CD4+ T helper epitope and flagellin/CpG1585 adjuvants induced strong and long-lasting CD8+ T cell responses and protected against ocular herpes. Building on the above published and preliminary data, we hypothesize that a SAPNs-based delivery system that incorporates human CD4+ and CD8+ T cell epitopes, recently identified in our lab from the VP16 and VP22 tegument proteins, can boost the number and function of protective TG- and OMS-resident CD4+ and CD8+ T cells and prevent or reduce recurrent ocular herpes disease. To test this hypothesis, we propose two Specific Aims: Aim 1: Test the hypotheses that therapeutic immunization of latently infected HLA double transgenic mice with SAPNs-based herpes vaccines delivering the VP16 and VP22 tegument proteins will boost HSV-specific TG- and OMS-resident CD4+ and CD8+ T cells and protect against UVB-induced recurrent ocular herpes infection and disease. Aim 2: Test the hypotheses that therapeutic immunization of latently infected HLA double transgenic mice with SAPNs- based herpes vaccines incorporating multiple pairs of immunodominant VP16 and VP22 CD4+ and CD8+ T cell epitopes will boost HSV-specific TG- and OMS-resident CD4+ and CD8+ T cells and protect against recurrent ocular herpes. Successful completion of the proposed work should help build a solid foundation toward developing an effective SAPNs-based ocular herpes vaccine.

PROJECT NARRATIVE

Potentially blinding recurrent ocular herpes disease, caused by HSV-1 infection, is a major global health problem. This proposal will test a novel Self Assembling Protein Nanoparticles (SAPNs)-based ocular herpes immunotherapy that deliver [redacted] The pre-clinical projet will use our [redacted] model of UVB-induced recurrent ocular herpes. Results from this preclinical study will pave the way toward developing a novel SAPNs-based ocular herpes immunotherapy for clinical applications.

HSV VP16 and VP22 tegument proteins or (2) recently identified human CD4+ and CD8+ T cell epitopes from the HSV VP16 and VP22 tegument proteins. The pre-clinical projet will use our recently developed “humanized” HLA transgenic (Tg) mouse

Facilities and Other Resources

This application benefits greatly from the collaboration between Sunomix Therapeutics, Inc and Dr BenMohamed laboratory of Cellular and Molecular Immunology at the UC Irvine School of Medicine by providing access to extensive and well-developed facilities (laboratory bench space, manufacturing space, [redacted] facilities, specialized equipment, technical support, computer systems, and office space) at multiple sites. This rich environment provides the necessary sophisticated facilities and resources to successfully complete the Specific Aims proposed in this application. As part of our ongoing collaboration, the investigative team has already established effective lines of scientific communication/interaction between the investigators at the different sites, together with established procedures for scientific study coordination and data interchange. Thus, we are strongly positioned to take optimal advantage of all available facilities immediately upon funding. Specific facilities, resources, and equipment to be made available to the proposed project are detailed below at each physical location: University of California Irvine as the primary applicant organization and Sunomix Therapeutic Inc., as the primary collaborating institution.

University of California Irvine

Laboratory: Research facilities are housed at the University of California Irvine main campus on the 2nd floor of Hewitt Hall, a state of the art research facility. Approximately 10,000 sq.ft. of space is dedicated for The Gavin Herbert Eye Institute (GHEI), of which over 4,000 sq. ft. is dedicated to herpes simplex virus research work. The PI's Laboratory of Cellular and Molecular Immunology at the GHEI has approximately 1200 sq. ft. with virology and immunology state-of-the-art equipment. This consists of all necessary equipment for the proposed project with the exception of high-end equipment, which is shared amongst other investigators at GHEI.

Clinics: Univ. of California Irvine main campus houses The Institute for Clinical and Translational Science (ICTS) facility, downstairs in the same Hewitt's Hall building as the PI's Lab upstairs, as well as many clinics that deals with patients with herpes infection and diseases. The PI has an approved facility to use blood from HSV seropositive symptomatic and asymptomatic individuals that are collected at UCI's ICTS or clinics. The PI's lab can be visited in this website: http://faculty.sites.uci.edu/benmohamedlab/

Office: The PI and lab members are linked through the campus Ethernet backbone via desktop Pentium computers. Each is equipped with word processing, data and statistical management, desktop publishing and presentation software.

The PI maintains an office in the GHEI of approximately 150 sq. ft. adjacent to the Laboratory of Cellular and Molecular Immunology. Eight under-graduate students, two senior technicians and 2 post-docs have desks and Pentium computers available in the lab proper.

Other: Our research labs have recently relocated from the University of California Medical Center to the main campus of the University of California Irvine. Our new labs are in one of the most highly desired newer research buildings on the main campus. All research equipment and activities expected at a major university are available. Our research laboratories are also staffed with a lab administrator and secretary. UCI provides a smoke free environment.

Sunomix Therapeutics, Inc.,

The R&D laboratory facilities for Sunomix Therapeutics, Inc, located into a 5,500 sq. ft. building at 6650 Sorrento Valley Rd, San Diego, CA 92121 building houses support staff for typical office functions, accounts and project management, grant management, and personnel records. Laboratory facilities available to conduct the studies proposed in this application include approximately 2,500 sq. ft. of wet bench space, 500 sq. ft. of tissue culture space, and 750 sq. ft. of equipment space. Included in the overall facility is office and general work space including conference rooms, lunch areas and open warehouse space. Facilities at Sunomix Therapeutics, Inc., fully support the R&D and production activities to be performed by the company in this proposal, including SAPNs construction, recombinant protein expression and purification, QA/QC assay and development.

Equipment

University of California Irvine

Major equipment available to the Cellular and Molecular Immunology Laboratory (LCMI), Gavin Herbert Eye Institute (GHEI), at UC Irvine main campus, includes the following: one Aria II 6 color Flow cytometer, one Luminex 100, one Confocal Microscope, a Caliper/Xenogen IVIS-100 imaging system, a Bio-Rad iMark Absorbance Microplate Reader with Microplate Manager 6 software, a Bio-Rad ELISA Microplate washer, and all necessary equipment for tissue culture and tissue staining including: 8 CO_2 and temperature controlled incubators, 4 BL2 Biosafety hoods, 2 chemical hoods, 4 microcentrifuges, 2 vacuum ovens, several temperature controlled water baths, various 4°/-20°C refrigerator/freezers, four -80°C freezers, 4 automated LN2 large capacity cell freezers, 2 high speed centrifuges, 1 cell harvester, 1 ultracentrifuge with rotors, 2 incubator-shaker for bacteria, scintillation counters (in the core facility), and laser imaging system (fluorescent and phosphorimaging)1 dark room with film developer and an enlarger, 2 fluorescence microscopes, 2 inverted microscopes with digital photographic equipment, 1 beta and 1 gamma scintillation counters, DNA sequencing equipment (in the core facility), 1 real time thermal cycler, 1 nucleic acid and protein electrophoresis and transfer. The laboratory is equipped with an Agillent 2100 Bioanalyzer, a Leica Laser Microdissection Station, 2 PCR machines, including real time PCR, 1 hybridization washing station, 2 hybridization ovens, manifolds and platforms for high throughput plasmid isolation and purification and a DNA sequencer.

Sunomix Therapeutics, Inc.,

Major lab equipment and facilities for SAPNs construction and protein expression and characterization are as follows: Controlled incubators with shaking platform, an oxygen generator, a GE Wave™ rocker with heat and oscillation control that supports 25 L fermentation runs and a M-110 microfluidizer with a dry-air compressor.

Added equipment and facilities for purifying, formulating, and characterizing products at Sunomix Therapeutics include: FPLC and HPLC chromatography systems (three analytical and one semi-preparative), a chromatography cabinet, a newly acquired Yamato spray dryer, a liposome extrusion apparatus, a light-scatter ultra-fine particle analyzer, an ultra-filtration apparatus, super-speed and ultra-speed centrifuges, a microfuge, a refrigerated table-top centrifuge, a roto-evaporator, sonicators (flow-through, probe, and bath), a UV. spectrophotometer, a bio-safety cabinet, laminar flow and chemical hoods, electrophoresis equipment, a CO2 incubator, an Axiovert inverted fluorescent microscope with digital camera, PCR equipment, a computer integrated Gel Documentation system, a compound microscope, and a full complement of freezers, refrigerators, and general lab equipment. Both lab and office space has a computer network providing software for word processing, relational databases, statistical programs, internet access, reference retrieval and Genebank/EMBL comparisons. All company facilities are secured by a card key entry system.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: LBACHIR Middle Name Last Name*: BENMOHAMED Suffix:

Position/Title*:	Professor/Director
Organization Name*:	The Regents of the University of California, Irvine
Department:	Ophthalmology Research
Division:	School of Medicine
Street1*:	Hewitt Hall Room 2032
Street2:	
City*:	Irvine
County:	Orange
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	92697-7600

Phone Number*: (949) 824-8937 Fax Number: (949) 824-9626

E-Mail*: lbenmoha@uci.edu

Credential, e.g., agency login: Lbenmohamed

Project Role*: PD/PI Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1997

Attach Biographical Sketch*: File Name: BiosketchBenMohamed1010649539.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12758901 Funding Opportunity Number: PA-18-489 . Received Date: 2018-12-10T19:07:05.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: CHRISTINE Middle Name Last Name*: MCLAREN Suffix:

Position/Title*: Professor
Organization Name*: The Regents of the University of California, Irvine
Department: Epidemiology
Division: School of Medicine
Street1*: Irvine Hall, Room 214
Street2:
City*: Irvine
County: Orange
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92697-7600

Phone Number*: (949) 824-4007 Fax Number: (949) 824-1343

E-Mail*: cmclaren@uci.edu

Credential, e.g., agency login: cmclaren

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Ph.D. Degree Year: 1983

Attach Biographical Sketch*: File Name: McLarenBio1010649540.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Mohammed Middle Name Last Name*: Bouziane Suffix:

Position/Title*: Chief Executive Officer
Organization Name*: Sunomix Therapeutics Inc
Department:
Division:
Street1*: 3210 Merryfield Row
Street2:
City*: San Diego
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92121-1126

Phone Number*: 858) 900-5059 Fax Number:

E-Mail*: mbouziane@sunomixtherapeutics.com

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1996

Attach Biographical Sketch*: File Name: BouzianeNIHBioSketch1010649532.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: PETER Middle Name Last Name*: BURKHARD Suffix:

Position/Title*: CEO
Organization Name*: Alpha-O Peptides AG
Department:
Division:
Street1*: Lörracherstrasse 50
Street2:
City*: 4125 Riehen
County:
State*:
Province:
Country*: CHE: SWITZERLAND
Zip / Postal Code*:

Phone Number*: +49 173 510 6736 Fax Number:

E-Mail*: peter.burkhard@aopeptides.ch

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PhD Degree Year: 1995

Attach Biographical Sketch*: File Name: BiosketchBurkhard1010649551.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

NAME: Lbachir BenMohamed

eRA COMMONS USER NAME: Lbenmohamed

POSITION TITLE: **Professor of Immunology**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	COMPLET DATE	FIELD OF STUDY
University Paris VII, Paris, France	B.S.	06/1990	Biochemistry
Pasteur Institute, Paris, France	M.S.	06/1991	Immuno-parasitology
Pasteur Institute & University Paris VII, Paris, France	Ph.D.	03/1997	Immunology
City of Hope National Medical Center, Duarte, CA	Post. Doc.	12/1998	Viral Immunology
Beckman Research Institute of Immunology, CA	Post. Doc.	12/2000	T cell Immunology

A. Personal Statement:

The goal of this new R21 grant application entitled "**PROTECTIVE IMMUNITY AGAINST RECURRENT OCULAR HERPES INDUCED WITH SELF-ASSEMBLING PROTEIN NANOPARTICLES"** is to pre-clinically test therapeutic ocular herpes vaccine candidate nanoparticles-based proteins (SAPNs) using a novel 6255 of UVB-induced recurrent ocular herpes. against induced HSV-1 reactivation from sensory ganglia, ocular shedding in tears and recurrent ocular herpetic disease. My lab has been working on herpes infection, immunity and immunopathology projects for over 20 years. I am the head and founder of Cellular and Molecular Immunology Laboratory at UC Irvine for last 15 years. Our team is recognized as a world leader in the fields of T-cell based ocular herpes vaccines and immunotherapies.

I am the principal investigator of this proposal and I am responsible for its conception in collaboration with one co-investigator, Dr. Bouziane Mohammed from Sunomix Therapeutics Inc., and other collaborators (Dr. Christine McLaren and Dr. Peter Burkhard). I will supervise the data collection and analysis and I will be involved in the data reporting. I have the expertise, leadership, and motivation to successfully carry out the proposed work. I have been the PI on successfully carried out NIH R03 and R01 grant projects. I have worked on cellular and molecular immunology of infectious diseases for over 25 years, beginning as a graduate and post doc at the Pasteur Institute (France). My lab has been at the forefront of ocular herpes immunology and immunotherapeutic vaccine research for 20 years and is internationally recognized as a leader in this field. I have published over 100 peer-reviewed papers, the majority in high impact journals, including *Nature Medicine, The Journal of Immunology, Mucosal Immunology*, *The Journal of Virology,* and *Investigative in Ophthalmology and Visual Sciences.*

For this project, I have gathered a multidisciplinary team with complementary expertise that includes top basic scientists from UC Irvine (Dr. BenMohamed, Dr. Christine McLaren and Dr. Prakash, Swayam) and Sunomix Therapeutics Inc., (Dr. Bouziane Mohammed and Dr. Peter Burkhard) with complementary expertise in protein synthesis proteomics, vaccine development against ocular herpes, T cell immunology, and animal models required for completion of this ocular herpes vaccine project. This multidisciplinary team will study ocular herpes immunity and immunopathology in 6255 potentially uncovering new molecular approaches to control HSV reactivation from latently and cure recurrent ocular herpes disease.

B. Positions and Honors:

Positions and Employment:

1998-1999 Post Doc, Dept. of Hematology/Bone Marrow transp., City of Hope Medical Center, CA.
1999-2000 Research Fellow: Dept. of Immunology. Beckman Research Institute, City of Hope, CA,

2001-2002 Scientist. Ophthalmology Research. Cedars-Sinai Medical Center, Los Angeles, CA.
2002-2007 Assistant Professor and Director Cellular Mol. Immunology Laboratory, UC Irvine, Irvine, CA
2007-2014 Associate Professor and Director Cellular Mol. Immunology Laboratory, UC Irvine, Irvine, CA
2014-present Full Professor & Director Cellular & Molecular Immunology Laboratory, UC Irvine, Irvine, CA
2016-present Scientist Immunologist, Sunomix Therapeutics, Inc., San Diego, CA

Other Experience and Professional Memberships:
2010-present NIH Reviewer National Institutes of Health (NIAID, NEI and NCI) Study Sections.
07-2010 NIH Reviewer, Member Conflict: Anterior Eye Disease (AED) Study Section [ZRG1].
02-2011 NIH Reviewer, Anterior Eye Disease (AED) Study Section.
06-2011 NIH Reviewer, NIH SBIR/STTR Grants, Small Business Diagnostic grants.
02-2012 NIH Reviewer, Strategies for the Protection of Pregnant Women (NIAID, ZAI1-BDP-M-M1).
06-2012 NIH Reviewer, Vaccines Against Microbial Diseases (VMD) Study section.
06-2013 NIH Reviewer, NIH Reviewer, Vaccines ZRG1 IMM N12 Study Section.
10-2013 NIH Reviewer, Vaccine Development and Immunology (ZRG1 IM-V) Study Section.
11-2013 NIH Reviewer, NIAID-DAIDS-NIH-AI-2012150, Immunology Quality Assessment Program.
02-2014 NIH Reviewer, Ad-hoc reviewer NIAID. Mucosal Environment (ZAI1 RB -A (J1) Study Section.
06-2014 NIH Reviewer, Immunology (ZRG1 IMM-N12) Study Section.
02-2015 NIH Reviewer, Diseases and Pathophysiology of the Visual System (DPVS) Study Section.
06-2015 NIH Reviewer, Special Emphasis Panel ZRG1 III-F 08 F, Innate Immunity and Inflammation.
06-2015 NIH Reviewer, Innate Immunity and Inflammation (III) Study Section.
07-2015 NIH Reviewer, Small Business: Non-HIV Microbial Vaccines ZRG1 IMM-R (12) Study Section.
10-2015 NIH Reviewer, Immunity and Host Defense (IHD) Study Section.
02-2016 NIH Reviewer, Cellular and Molecular Immunology (CMIA) Study Section.
03-2016 NIH Reviewer, Special Emphasis Panel ZRG1-BDCN-N-55, Study Section.
05-2016 NIH Reviewer, Special Emphasis Panel ZRG1-BDCN-W-90 Study Section.
06-2016 NIH Reviewer, Cellular and Molecular Immunology (CMIA) Study Section.
02-2017 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-J-81) Study Section.
02-2017 NIH Reviewer, Immunity and Host Defense (IHD) Study Section.
03-2017 NIH Reviewer, Immunology (ZRG1-IMM-C-02) Study Section.
06-2017 NIH Reviewer, Innate Immunity and Inflammation (III) Study Section.
10-2017 NIH Reviewer, Clinical Neuroimmunology and Brain Tumors Study Section (CNBT) Study Section.
10-2017 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-J-81) Study Section.
11-2017 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-R-03) Study Section.
03-2018 NIH Reviewer, Special Emphasis Panel ZRG1-BDCN-W-90 Study Section.
03-2018 NIH Reviewer, Ocular Surface, Cornea, Anterior Segment (ZRG1-BDCN-J-81) Study Section.
04-2018 NIH Reviewer, Member Conflict: Topics in Virology (ZRG1 IDM-W-02) Study Section.
06-2018 NIH Reviewer, Clinical Trials (ZAI1-MFH-M-S2) Study Section.
06-2018 NIH Reviewer, Cellular and Molecular Immunology (CMIA) Study Section.
09-2018 NIH Reviewer, Lung Cellular, Molecular, and Immunobiology (LCMI) Study Section.

Honors:
1992-1996 Fellowship from the French Government, France
1996-1997 Fellowship from Pasteur Institute, Paris, France
1998 Award from American Society of Hematology, USA
1999 Award from American Society of Hematology, USA
2006; Award from Research to Prevent Blindness (RPB), New York, USA
2009, 2010 and 2014, 2018 Award from the Discovery Fund for Eye Research, Los Angeles, CA, USA

C. Contribution to Science:

Dr. BenMohamed's work has been highly influential in shaping the current understanding of herpes T cell-mediated immunity in both humans [redacted]: (**1**) He developed mucosal delivery of clinically approved lipopeptide vaccines and immunotherapies to protect against herpes infection and disease. (**2**) He recently introduced a novel concept of symptomatic/asymptomatic immunology to defined the underlying mechanisms by which T cells specific to asymtomatic epitopes protect against herpes. (**3**) He discovered new immune evasion mechanisms by which HSV-1 LAT gene interferes with T cell immunity. (**4**) He developed a novel [redacted]6255 model of genital herpes (a

model used in this proposal). (**5**) Finally, his lab has identified many HSV-1 and HSV-2 human $CD4^+$ and $CD8^+$ T cell epitopes for vaccine and immunotherapy purposes.

These five major contributions to science are detailed below:

1. Developed mucosal delivery of clinically approved vaccines and immunotherapies to protect against herpes infection and disease: Targeting of the genital mucosal immune system with subunit vaccines has failed to induce potent and durable local $CD8^+$ T cell immunity, which is crucial for protection. Dr. BenMohamed is the key developer and co-inventor of a new promising vaccine strategy that uses mucosal delivery of clinically approved lipopeptide vaccine molecules, laser adjuvant vaccine, and recently prime/pull vaccine strategy. Many researchers have now successfully tested these vaccine strategies, around the world, to protect against many infectious mucosal pathogens.

a. Phenotypic and Functional Signatures of Herpes Simplex Virus-Specific Effector Memory $CD73^+CD45RA^{high}CCR7^{low}CD8^+$ T_{EMRA} and $CD73^+CD45RA^{low}CCR7^{low}CD8^+$ T_{EM} Cells are Associated with Asymptomatic Ocular Herpes. Srivastava, R. & **BenMohamed L**. *The Journal of Immunology*. **2018**. *In press.*

b. CXCL17 Chemokine–Dependent Mobilization of CXCR8+ CD8+ Effector Memory and Tissue-Resident Memory T Cells in the Vaginal Mucosa Is Associated with Protection against Genital Herpes. Srivastava, R., Hernandez-Ruiz, M., Khan, A.A. Fouladi, M.A., Kim, G.J., Ly, V.T., Yamada, T., Lam, C., A. Sarain, S.A., Boldbaatar, U., Zlotnik, A., Bahraoui, E. & **BenMohamed L**. *The Journal of Immunology*. **2018**. 200(8):2915-2926. **PMID: 29438765**.

c. Bolstering the Number and Function of HSV-1-Specific $CD8^+$ Effector Memory T Cells and Tissue-Resident Memory T Cells in Latently Infected Trigeminal Ganglia Reduces Recurrent Ocular Herpes Infection and Disease. Khan AA, Srivastava R, Chentoufi AA, Kritzer E, Chilukuri S, Garg S, Yu DC, Vahed H, Huang L, Syed SA, Furness JN, Tran TT, Anthony NB, McLaren CE, Sidney J, Sette A, Noelle RJ, & **BenMohamed L**. *The Journal of Immunology*. **2017**. 199(1):186-203. **PMID: 28539429**.

d. A genital tract peptide epitope vaccine targeting TLR-2 efficiently induces local and systemic CD8+ T cells and protects against herpes simplex virus challenge. Zhang X, Chentoufi AA, Dasgupta G, Nesburn AB, Wu M, Zhu X, Carpenter D, Wechsler SL, You S, & **BenMohamed L.** *Mucosal Immunology*. (*Nature Publishing Group*). **2009.** 2(2):129-43. **PMID: 19129756**.

2. Discovered a novel "*asymptomatic memory $CD8^+$ T cells concept*" in herpes immunity: Generation and maintenance of high quantity and quality memory $CD8^+$ T cells determine the level of protection from viral, bacterial, and parasitic re-infections, and hence constitutes a primary goal for T cell epitope-based human vaccines and immunotherapeutics. Dr. BenMohamed recently introduced a new direction in developing T cell-based human herpes vaccines and immunotherapeutics based on the emerging new concept of "*asymptomatic memory $CD8^+$ T cells"*. For this he categorized the phenotype, the function and the anatomical locations of two new major distinct sub-populations of memory symptomatic and asymptomatic HSV-specific $CD8^+$ T cells based on their protective vs. pathogenic function. Several asymptomatic HSV human epitopes have been since identified in Dr. BenMohamed's laboratory and are currently considered for T cell-based human herpes "asymptomatic" vaccine.

a. [redacted]

b. Phenotypic and Functional Characterization of Herpes Simplex Virus Glycoprotein B Epitope-specific Effector and Memory CD8+ T Cells from Ocular Herpes Symptomatic and Asymptomatic Individuals. Arif Azam Khan; Ruchi Srivastava; Doran Spencer; Daniel Fremgen; Hawa Vahed; Patricia P. Lopes; Thanh T Pham; Charlie Hewett; Jasmine Kuang; Nicolas Ong; Lei Huang;

Vanessa M. Scarfone, Anthony B. Nesburn; Steven L. Wechsler & **BenMohamed L.** *The Journal of Virology*. **2015**. 89(7): 3776-92. **PMID: 25609800.**

c. Asymptomatic HLA-A*02:01-restricted epitopes from herpes simplex virus glycoprotein B preferentially recall polyfunctional CD8+ T cells from seropositive asymptomatic individuals and protect 6255 against ocular herpes. Dervillez X, Qureshi H, Chentoufi AA, Khan AA, Kritzer E, Yu DC, Diaz OR, Gottimukkala C, Kalantari M, Villacres MC, Scarfone VM, McKinney DM, Sidney J, Sette A, Nesburn AB, Wechsler SL, & **BenMohamed L**. *The Journal of Immunology*. **2013** 15;191(10):5124-38. **PMID: 24101547**.

d. Immunodominant "asymptomatic" herpes simplex virus 1 and 2 protein antigens identified by probing whole-ORFome microarrays with serum antibodies from seropositive asymptomatic versus symptomatic individuals. Dasgupta G, Chentoufi AA, Falatoonzadeh P, Chun S, Lim CH, Felgner PL, Davies DH, & **BenMohamed L**. *The Journal of Virology*. **2012**. 86(8):4358-69. **PMID: 22318137**.

3. Discovered exhaustion as a novel immune evasion mechanism of HSV-specific CD8+ T cells, a mechanism that is induced by herpes LAT gene expressed during herpes latency: We demonstrated, for the first time, in both mouse and rabbit model of herpes infection that most of the HSV-1-specific CD8+ T cells that are selectively retained in sensory ganglia, the site of latent infection, were phenotypically and functionally exhausted. In this novel immune evasion mechanisms, HSV-1 LAT gene promotes functional exhaustion (i.e., dysfunction) of HSV-specific CD8+ T cells resulting in virus reactivation.

a. [redacted]

b. The Herpes Simplex Virus Type 1 Latency Associated Transcript Inhibits Phenotypic and Functional Maturation of Dendritic Cells. Chentoufi, AA., Dervillez, X., Dasgupta G., Nguyen C., Kabbara, KW., Jiang X., Nesburn, A.B., Wechsler S.L. & **BenMohamed L.** *Viral Immunology*. **2012**. *(3): 204-15.* **PMID: 22512280.**

c. The Herpes Simplex Virus-1 Encoded Latency-Associated Transcript Promotes Dysfunctional Virus-Specific CD8+ T Cells in Latently Infected Trigeminal Ganglia: A Novel Immune Evasion Mechanism. Chentoufi, A.A., E. Kritzer, M. Tran, G. Dasgupta, R. EA., J. Xianzhi, D. Carpenter, O. Osorio, A. B. Nesburn, L. Wechsler & **BenMohamed, L**. *The Journal of Virology*. **2011**. 85(17): 9127-38. **PMID: 21715478.**

d. The herpes simplex virus type 1 latency-associated transcript can protect neuron-derived C1300 and Neuro2A cells from granzyme B-induced apoptosis and CD8 T-cell killing. Jiang X[1], Chentoufi AA, Hsiang C, Carpenter D, Osorio N, **BenMohamed L**, Fraser NW, Jones C, Wechsler SL. *The Journal of Virology*. **2011**. 85(5): 2325-32. **PMID: 21177822.**

[redacted]

a. [redacted]

b.

c. [redacted]

d. [redacted]

5. Leader in mapping of human CD4⁺ and CD8⁺ T cell epitopes from HSV-1 protein antigens for ocular herpes vaccine and immunotherapy purposes: Dr. BenMohamed's efforts in last 2 decades had let to identification of several CD4⁺ and CD8⁺ T cell epitopes from many herpes glycoprotein and tegument proteins that are currently being considered fro clinical herpes vaccine trials.

a. HLA-A02:01-restricted epitopes identified from the herpes simplex virus tegument protein VP11/12 preferentially recall polyfunctional effector memory CD8+ T cells from seropositive asymptomatic individuals and protect humanized 6255 against ocular herpes. Srivastava R, Khan AA, Spencer D, Vahed H, Lopes PP, Thai NT, Wang C, Pham TT, Huang J, Scarfone VM, Nesburn AB, Wechsler SL. & **BenMohamed L**. *The Journal of Immunology*. **2015**. 194(5): 2232-48. **PMID: 25617474**.

b. Asymptomatic HLA-A*02:01-restricted epitopes from herpes simplex virus glycoprotein B preferentially recall polyfunctional CD8+ T cells from seropositive asymptomatic individuals and protect 6255 against ocular herpes. Dervillez X1, Qureshi H, Chentoufi AA, Khan AA, Kritzer E, Yu DC, Diaz OR, Gottimukkala C, Kalantari M, Villacres MC, Scarfone VM, McKinney DM, Sidney J, Sette A, Nesburn AB, Wechsler SL. & **BenMohamed L**. *The Journal of Immunology*. **2013**. 191(10): 5124-38. **PMID: 24101547**.

c. HLA-A*0201-restricted CD8+ cytotoxic T lymphocyte epitopes identified from herpes simplex virus glycoprotein D. Chentoufi AA, Zhang X, Lamberth K, Dasgupta G, Bettahi I, Nguyen A, Wu M, Zhu X, Mohebbi A, Buus S, Wechsler SL, Nesburn AB. & **BenMohamed L**. *The Journal of Immunology*. **2008**. 180(1): 426-437. **PMID: 18097044**.

d. Asymptomatic human CD4+ cytotoxic T-cell epitopes identified from herpes simplex virus glycoprotein B. Chentoufi AA, Binder NR, Berka N, Durand G, Nguyen A, Bettahi I, Maillère B., & **BenMohamed, L**. *The Journal of Virology*. **2008**. 82(23): 11792-802. **PMID: 18799581.**

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Benmohamed

D. Ongoing Research Support:

1. [redacted]
2. R01 EY026103-01A1. (**BenMohamed, PI**). Mechanisms of CD8⁺ T Cell Dynamics in Recurrent Ocular Herpetic Disease. NIH/NEI Period: **04/01/16 - 03/31/2020.**
3. R41 AI138764-01 (**BenMohamed, PI**). A Novel Self-Assembling Protein Nanoparticles-Based Genital Herpes Vaccine. NIH/NIAID Period: **07/01/18 - 06/30/2019.**

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

NAME: McLaren, Christine E.

eRA COMMONS USER NAME (credential, e.g., agency login): cmclaren

POSITION TITLE: Professor of Biostatistics

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
California State University, San Jose, CA	BS (Honors)	06/69	Mathematics
Stanford University, Palo Alto, CA	MA	06/70	Mathematics Education
Case Western Reserve University, Cleveland, OH	MS	06/76	Mathematical Statistics
Case Western Reserve University, Cleveland, OH	PhD	06/83	Biostatistics

A. Personal Statement. I am Professor and Vice Chair, Department of Epidemiology and I am Interim Chair of the Biostatistics Shared Resource, Chao Family Comprehensive Cancer Center (CFCCC). I have over 25 years of experience in the design, conduct, and statistical analysis of research studies. I have focused on statistical modeling research that provides insight into biological processes distinguishing between health and disease. In 1993, I was elected a Fellow of the American Statistical Association, in part for “innovative research in biology and medicine”.

I have a longstanding and successful working relationship Dr. BenMohamed. For this project, I will provide analysis of immune responses to ocular herpes immunotherapy IN 6255 I will provide detailed descriptive and analytic reports and will participate in abstract and manuscript preparation.

B. Positions and Honors

Positions and Employment

1976-79 Research Biostatistician, Department of Biometry, Case Western Reserve University
1979-80 Research Officer, Department of Haemotology, Welsh National School of Medicine
1980-83 Research Biostatistician, Department of Biometry, Case Western Reserve University
1983-84 Senior Instructor, Department of Biometry and Department of Medicine (Cleveland Metropolitan General Hospital), Case Western Reserve University
1984-86 Assistant Professor, Department of Biometry and Department of Medicine (Cleveland Metropolitan General Hospital), Case Western Reserve University
1986-87 Assistant Professor, Department of Mathematics, Minnesota State University Moorhead
1987-92 Associate Professor, Department of Mathematics, Minnesota State University Moorhead
1992-98 Professor, Department of Mathematics, Minnesota State University Moorhead
1998-present Professor of Medicine (Epidemiology) and Director of Biostatistics (Chao Family Comprehensive Cancer Center), University of California, Irvine
2008-present Vice Chair for Academic Affairs, Department of Epidemiology, University of California, Irvine

Other Experience and Professional Memberships

1984-2002 International Committee for Standardization in Hematology (Cytometry), Statistical Consultant
1990, 1999 National Science Foundation, Division of Mathematical Sciences, Grant Review Panel
1994, 2000-04 National Institutes of Health, Statistical Reviewer, Hematology Study Section, CSR
2001-2004 Veterans Health Administration, Member, Epidemiology Merit Review Subcommittee
2005-2006 NIH, Ad-hoc Reviewer, NCI Clinical Oncology Study Section
2006 NIH, NCI Initial Review, NCI-A RTRB-H (L1), Subcommittee A – Cancer Centers
2007 NIH Ad-hoc Reviewer, Subcommittee 1-Career Development
2007-2011 NIH, Member, NCI Clinical Oncology (CONC) Study Section
2012 NCI, Reviewer, SPORE in Breast, Endometrial, and Skin Cancers, ZCA1 RPRB-0 M1 P
2013 NCI Oncology 2 - Translational Clinical Integrated Review Group

2014 NCI, Reviewer, P01 Special Emphasis Panel III, ZCA1 RPRB-0 (J1)
2015 NCI, Reviewer, Special Emphasis Panel, ZCA1 PCRB-C (M1) R
2016 NCI Specialized Programs of Research Excellence (SPORE) Review Group
2016 NCI, Chair of Omnibus SEP16 R03 & R21 Review Group, 2016/05 ZCA1 PCRB-C (M1) S
2018 NCI Specialized Programs of Research Excellence (SPORE) Review Groups

Honors

1983-84 American Heart Association Research Fellowship
1985 Visiting Scientific Officer, University of Wales College of Medicine, United Kingdom
1986-present Fellow, Royal Statistical Society
1991 Senior Honorary Research Fellowship, University of Glasgow, United Kingdom
1993 Phi Kappa Phi Honor Society
1993-present Fellow, American Statistical Association
1994-1995 Raybould Visiting Fellowship, Dept. of Mathematics, Univ. of Queensland, Brisbane, Australia
1995 Senior International Fellowship awarded by the NIH Fogarty International Center
1996 University Dean's Council Nominee, 1997 US Professors of the Year Program, Carnegie Foundation for the Advancement of Teaching
2004 American Statistical Association Service Award, Council of Chapters
2013 Clinical and Translational Science (ICTS) Interdisciplinary Team Science Award, Athena Breast Health Network Program, University of California, Irvine
2014 "Best of ASH" award, 56th meeting of the American Society of Hematology, Dec. 5-9, 2015

C. Contributions to Science

1. **Collaborative Research in Cancer**. My collaborative efforts in optical and magnetic imaging are illustrated by my participation as a co-investigator and lead biostatistician for multiple grants. I have co-authored publications resulting from studies of dynamic contrast-enhanced magnetic resonance imaging as a clinical imaging modality for the detection, diagnosis, and treatment of breast lesions. As an example, I supervised statistical modeling using generalized estimating equations (GEE) models that incorporated therapy response, treatment regimen, measurement day, and interaction terms to assess the outcomes of oxyhemoglobin, deoxyhemoglobin, water, and lipid. The results showed that functional hemodynamic and metabolic information acquired using a noninvasive optical imaging method on the first day after neoadjuvant chemotherapy treatment can discriminate nonresponding from responding patients. As Director of the Data Coordinating Center for NIH/NCI grant R01 CA88078-01 (F.L. Meyskens, P.I.), I provided analyses and interpretation of data from the landmark study that demonstrated that recurrent adenomatous polyps can be markedly reduced by a combination of low oral doses of difluoromethylornithine and sulindac and with few side effects.

   a. **McLaren CE**, Fujikawa-Brooks S, Chen W-P, Gillen DL, Pelot D. Gerner EW, Meyskens FL. Longitudinal assessment of air conduction audiograms in a phase III clinical trial of DFMO and sulindac for prevention of sporadic colorectal adenomas. Cancer Prev Res 1:514-521, 2008. PMC2702261.
   b. **McLaren CE**, Chen W-P, Nie K, Su M-Y. Prediction of malignant breast lesions from MRI features: a comparison of artificial neural network and logistic regression techniques. Acad Radiol 16(7):842-51, 2009. PMC2832583
   c. Roblyer D, Ueda S, Cerussi A, Tanamai W, Durkin A, Mehta R, Hsiang D, Butler JA, **McLaren C**, Chen WP, Tromberg B. Optical imaging of breast cancer oxyhemoglobin flare correlates with neoadjuvant chemotherapy response one day after starting treatment. Proc Natl Acad Sci USA 108(35):14626-31, 2011. PMC3167535**.**
   d. O'Sullivan TD, Leproux A, Chen JH, Bahri S, Matlock A, Roblyer D, **McLaren CE**, Chen WP, Cerussi AE, Su MY, Tromberg BJ. Optical imaging correlates with magnetic resonance imaging breast density and reveals composition changes during neoadjuvant chemotherapy. Breast Cancer Res 15(1):R14, 2013. PMC3672664.

2. **Hemochromatosis and Iron Overload**. Hemochromatosis is a hereditary disease in which affected persons suffer excessive dietary iron absorption and may lead to complications such as liver cirrhosis, hepatocellular carcinoma, heart failure, diabetes, arthritis, and impotence. I have 24 years of experience working on hematological studies and have published methodological and applied papers related to hemochromotosis,

iron overload, and measures of iron status. As Principal Investigator of a Field Center for the Hemochromatosis and Iron Overload Screening (HEIRS) Study, I was lead author on the initial paper describing the overall study design. I supervised enrollment of 20,400 participants at the University of California, Irvine. Based on data from 99,711 participants, we found that the C282Y (substitution of tyrosine for cysteine at amino acid 282) mutation of the *HFE* gene is most common in whites and is accompanied by elevations on iron measures. As a co-investigator for The Melbourne Collaborative Cohort Study, I was co-author of papers describing results from the prospective cohort in which participants born in Australia, New Zealand, the United Kingdom, or Ireland (n=28,509) were genotyped for the *HFE* C282Y variant. Iron-overload-related disease developed in a substantial proportion of C282Y homozygous men. *HFE* C282Y homozygotes have twice the risk of colorectal and breast cancer compared with those individuals without the C282Y variant.

a. **McLaren CE**, Barton JC, Adams PC, Harris EL, Acton RT, Press N, Reboussin DM, McLaren GD, Sholinsky P, Walker AP, Gordeuk VR, Leiendecker-Foster C, Dawkins FW, Eckfeldt JH, Mellen BG, Speechley M, Thomson E for the Hemochromatosis and Iron Overload Study Research Investigators. Hemochromatosis and iron overload screening (HEIRS) Study Design for an Evaluation of 100,000 primary care-based adults. The Am J Med Sci 325:53-62, 2003. PMID: 12589228.
b. **McLaren CE**, Gordeuk VR, Chen WP, Barton JC, Acton RT, Speechley M, Castro O, Adams PC, Snively BM, Harris EL, Reboussin DM, McLachlan GJ, Bean R. Bivariate mixture modeling of transferrin saturation and serum ferritin concentration in Asians, African Americans, Hispanics, and Whites in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Trans Res 151(2):97-109, 2008. PMC3785302.
c. Osborne NJ, Gurrin LC, Allen KJ, Constantine CC, Delatycki MB, **McLaren CE**, Gertig DM, Anderson GJ, Olynyk JK, Powell LW, Hopper JL, Giles GG, English DR. HFE C282Y homozygotes are at increased risk of breast and colorectal cancer. Hepatology 51(4):1311-8, 2009. PMC3815603.

3. **Genetic Components of Iron Status**. As PI **OF** NIH grant R01-HL083328-01A1, “Iron Status: A Pathway Analysis in Multiple Ethnicities”, I led a multi-center project to study the heritability of serum iron measures, determine single nucleotide polymorphisms (SNPs) and haplotypes in key genes involved in systemic iron metabolism pathways, identify potential cases of iron deficiency and controls, and study the association between the presence of iron deficiency and haplotypes in the selected candidate genes. Heritability is the proportion of observed variation in a trait among individuals in a population that is attributable to hereditary factors. Participants (N=942) were 77% Caucasians, 10% Asians, 8% Hispanics, and 5% other race/ethnicities. We found that serum iron measures have significant heritability components, after excluding known genetic and nongenetic sources of variation**.** Subsequently, we performed a genome-wide association study (GWAS) using DNA collected from participants in the HEIRS Study to identify new genomic locations associated with iron deficiency. Replication analyses were performed in a sample of veterans screened at a US Veterans Affairs (VA) medical center. The joint analysis of the HEIRS and VA samples revealed strong associations between rs2698530 on chr. 2p14 and iron status outcomes, confirming a previously-described *TF* polymorphism and implicating one potential new locus as a target for gene identification**.** A follow-up study of white, African-American, Hispanic, and Asian HEIRS participants analyzed the association between SNPs and eight iron-related outcomes. Three chromosomal regions showed association across multiple populations, including SNPs in the *TF* and *TMPRSS6* genes, and on chromosome 18q21. A novel SNP rs1421312 in *TMPRSS6* was associated with serum iron in whites ($P=3.7x10^{-6}$) and replicated in African Americans ($P = 0.0012$). Our results confirmed known associations with iron measures and gave unique evidence of their role in different ethnicities, suggesting origins in a common founder. I am currently the PI of a separate multi-site NIH grant 1R24 DK099846-01A1, “Genetic Modifiers of Iron Status in Hemochromatosis *HFE* C282Y Homozygotes”. We hypothesized that variants of genes other than *HFE* and those previously associated with hemochromatosis and iron overload phenotypes are involved in the regulation of iron metabolism and modulate expression of iron overload in *HFE* C282Y homozygotes. We studied *HFE* C282Y homozygotes at the extremes of phenotypic expression and determined that *GNPAT* p.D519G is associated with a high-iron phenotype in *HFE* C282Y homozygotes and may participate in hepcidin regulation.

a. **McLaren CE**, Barton JC, Eckfeldt JH, McLaren GD, Acton RT, Adams PC, Henkin LF, Gordeuk VR, Vulpe CD, Harris EL, Harrison BW, Reiss JA, Snively BM. Heritability of Serum Iron Measures in the Hemochromatosis and Iron Overload Screening (HEIRS) Family Study, Am J Hematol 85(2):101-5, 2010**.** PMC3816512.

b. **McLaren CE**, Garner CP, Constantine CC, McLachlan S, Vulpe CD, Snivelely BM, Gordeuk VR, Nickerson DA, Cook JD, Leiendecker-Foster C, Beckman KB, Eckfeldt JH, Barcellos LF, Murray JA, Adams PC, Acton RT, Killeen AA, McLaren GD. James D. Genome-wide association study identifies genetic loci associated with iron deficiency. PLoS ONE 6(3):e17390, 2011. PMC3069025.
c. **McLaren CE**, McLachlan S, Garner CP, Vulpe CD, Gordeuk VR, Eckfeldt JH, Adams PC, Acton RT, Murray JA, Leiendecker-Foster C, Snively BM, Barcellos LF, Cook JD, McLaren GD. Associations between single nucleotide polymorphisms in iron-related genes and iron status in multiethnic populations. PLoS One 7(6):e38339, 2012. PMC3382217.
d. **McLaren CE**, Emond MJ, Subramaniam N, Phatak PD, Barton JC, Adams PC, Goh JB, McDonald CJ, Powell LW, Gurrin LC, Allen KJ, Nickerson DA, Louie T, Ramm, GA, Anderson GJ, McLaren GD. Exome sequencing in HFE C282Y homozygous men with extreme phenotypes identifies a GNPAT variant associated with severe iron overload. Hepatology 62(2):429-439, 2015**.** PMC450823.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/christine.mclaren.1/bibliography/44197942/public/?sort=date&direction=descending

D. Research Support
Ongoing Research Support

5R01 EY026103-02 (L. BenMohamed, PI) 08/01/16-7/31/20
Mechanisms of CD8$^+$ T Cell Dynamics in Recurrent Ocular Herpetic Disease
This is mechanistic and translational preclinical research of recurrent ocular herpes disease, caused by HSV-1 infection, designed to develop a clinical T-cell based immunotherapy against recurrent ocular herpes.
Role: Co-investigator

5R01CA195466- 02 (B. Tromberg, PI) 03/01/16-02/28/19
Quantitative Multiphoton Microscopy for Non-invasive Diagnosis of Melanoma
This purpose of this proposal is to evaluate the ability of in vivo multiphoton microscopy to reliably distinguish between pigmented lesions in three groups: common nevi, atypical nevi and melanoma.
Role: Co-investigator

1 R21 HL145232-01 CE McLaren (PI) 09/15/18-08/31/20
NIH/NHLBI
“Modulation of Iron Overload by Hepcidin and Erythroferrone”
This research will conduct a collaborative study to characterize the utility of serum hepcidin concentration and erythroferrone in identifying hemochromatosis patients who are at greatest risk of developing severe iron overload.
Role: PI

5 R24 DK 099846-03 CE McLaren/GD McLaren (PIs) 09/01/14-06/31/19
NIH/NIDDK
“Genetic Modifiers of Iron Status in Hemochromatosis HFE C282Y Homozygotes”
This research is to conduct a collaborative study that will answer the question, "What role do genetic modifiers play in determining iron accumulation in persons homozygous for the HFE C282Y genotype?"
Role: PI

1R21 CA208938 (PI: Su, M-Y) 08/01/17 – 7/31/19
NIH/NCI
Mammographic Density and Metabolic Genotyping for Predicting Cancer Prognosis”
This project will investigate the role of quantitative mammographic density (MD) and cytochrome P450 CYP2D6 metabolic genotyping in predicting the prognosis of breast cancer patients with hormonal receptor positive breast cancer receiving Tamoxifen treatment.
Role: Co-Investigator

2 P30 CA 062203-20, Van Etten, R. (PI) 09/11/97-01/31/21
NIH/NCI

"Cancer Center Support Grant"
The Cancer Center Support Grant provides support for administration and infrastructure for the UC Irvine Chao Family Comprehensive Cancer Center. Dr. McLaren is co-Leader of the Program in Cancer Control and Interim Interim Director of the Biostatistics Shared Resource.
Role: Co-Investigator

1DP7OD020321-01 (D. Fruman, PI) 09/18/15-08/31/19
UCI-GPS: UC Irvine Graduate Professional Success
This is an effort at UC Irvine to prepare PhD students and postdoctoral fellows for a variety of career options.
Role: Co-Investigator

5R01 CA142989-07 (B. Tromberg, PI) 01/01/10-06/30/18
Developing DOSI Technology for Monitoring Response of Breast Cancer Chemotherapy
Diffuse optical spectroscopic imaging is developed for clinical translational studies to monitor and predict pre-surgical neoadjuvant chemotherapy response early in treatment.
Role: Co-Investigator

5R01 EY019896-07 (L. BenMohamed, PI) 09/01/10-03/31/20

[redacted]

2P30 CA062203-19 (R. Van Etten, PI) 02/01/09-01/31/21
Cancer Center Support Grant
This grant supports the NCI-designated Chao Family Comprehensive Cancer Center at UC Irvine.
Role: Co-Investigator

Completed Research Support (selected)

1R21 CA166839-01A1 (M. Lilly and Z. Zi, MPI) 09/01/13-08/31/15
Phase 1 bioassay-guided Trial of Lycopene and Docetaxel for Prostate Cancer
This research will perform a Phase I trial of lycopene in combination with docetaxel as first-line chemo-therapy for patients with castration-resistant prostate cancer.
Role: Co-investigator

1R21 CA170955-01A1 (M-Y Su, PI) 01/15/13–01/14/15
Volume and Morphology of Fibroglandular Tissue for Breast Cancer Risk
This project will evaluate the role of MRI-based density parameters, including the volume and the morphology of the fibroglandular tissue, and build a risk prediction model using a case-control study design.
Role: Co-investigator

R24 DK093433-01 (C. McLaren, PI) 08/15/11-08/31/14
Genetic Modifiers of Iron Status in Hemochromatosis HFE C282Y Homozygotes
This research is to conduct a collaborative study that will answer the question, "What role do genetic modifiers play in determining iron accumulation in persons homozygous for the *HFE* C282Y genotype?"
Role: PI

N01 CN35160 (F. Meyskens, PI) 09/30/03-09/29/14
Phase 1 & 2 Clinical Trials of Chemoprevention Agents-Cancer
The overall objectives of this proposal are to conduct multiple early phase (Phase 1 and Phase 2) clinical trials of candidate cancer preventive agents and to assess the effect of compounds on biological or imaging endpoints.
Role: Co-Investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Mohammed Bouziane

eRA COMMONS USER NAME (credential, e.g., agency login): mbouziane1967

POSITION TITLE: CEO

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
RENE DESCARDES, UNIVERSITY PARIS V, FRANCE	M.S	09/1992	Molecular & Cellular Pathology
UNIVERSITY PARIS XI, IFSBM, FRANCE	Biomedical	09/1996	Biomedical/Pharmaceuticals
Gustave Roussy Institute & RENE DESCARDES, UNIVERSITY PARIS V, FRANCE	Ph.D.	09/1996	Molecular Genetics
City of Hope National Medical Center Beckman Research Institute Duarte, CA	Post. Doc.	09/1999	Mutagenesis/Genomics

A. Personal Statement:

Executive biopharmaceutical professional with over 20 years of academic & industry experience in advanced development of biomarkers, therapeutics pipeline, cutting edge innovative products and technologies. Extensive experience in leading multi-disciplinary groups from Discovery, R&D to preclinical, and early clinical programs. Strong and broad scientific expertise covering multiple areas of genomics, proteomics, mutagenesis, diagnostics, vaccines and cell therapies for the treatment of infectious diseases, cancer and immunotherapies. I have led several preclinical and IND-enabling developments based on different viral vector delivery systems (lentivirus, AAV, and adenovirus vectors) and non-viral delivery systems (DNA-based systems, electroporation) ranging from HIV therapeutic vaccine to treatment of infectious diseases. I have a strong publication record in applied and basic research published in high impact factor magazines including JBC, MCB, Clin Immuno, NAR, JMB, MAD, Mutat Res, Act Biochim, Am J Clin, Eu J Bio, JBSD, Biochemistry.

As a co-investigator on your new HSV nanoparticle-based ocular herpes therapeutic vaccine project, I will be responsible for the design and construction of the Self Assembling Protein Nanoparticles (SAPNs) you proposed in your new STTR proposal. As a business partner, Sunomix Therapeutics, Inc. will provide you self-assembling protein nanoparticles (SAPNs) that will incorporate your recently discovered UL48 and its human CD4 and CD8+ T cell epitopes of HSV-1. I will direct the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for HSV vaccine. Your preliminary data showing the immunogenicity and protective efficacy of your prototype SAPNs vaccine in your UVB-induced recurrent corneal herpes disease HLA double transgenic mouse model are very promising. Successful completion of the proposed work using [redacted] Developing vaccines is the core business of Sunomix Therapeutics. I believe that I am qualified to be the lead co-investigator in your ocular herpes therapeutic vaccine project.

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B. Positions and Honors:

Positions and Employment:

1996-1999 Post Doc, Dept. of Cancer, Mutagenesis, DNA Repair., City of Hope Medical Center, Beckman Research Institute, Duarte, CA.
1999 Job Offer, promotion to Assistant Professor
1999-2005 Group Leader, Scientist: Custom Technology Team. Becton Dickinson, San Diego, CA
2005-2010 Chief Scientific Officer, Biotox Sciences, San Diego, CA.
2010-present CEO,Sunomix Therapeutics, San Diego CA

Honors:

1993-1996 Fellowship from the French Government, IFSBM France
1996 Outstanding award from Cancer Research Association, Paris, France
1999 Special Award from City of Hope Medical Center, Beckman Research Institute, Duarte, CA. USA
2003 Award from Becton Dickinson, USA
2004 Special Award from Sedac Therapeutics, Geneva.
2005 Certified ISO9000 Auditor from Global Quality System, CA
2006 Award Economic Ambassador from Fincome, Morocco

C. Contribution to Science:

Dr. Bouziane's work has been highly influential in developing cutting edge innovative products and technologies in the biopharmaceutical & academic industry.

These five major contributions to science are detailed below:

1. **DNA triple helix-mediated inhibition of HIV-1 U5 long terminal repeat integration in vitro.**

Integration of the human immunodeficiency virus (HIV) DNA into the host genome is an obligatory process in the replicative life cycle of the virus. This event is mediated in vitro by integrase, a viral protein which binds to specific sequences located on both extremities of the DNA long terminal repeats (LTRs). These sites are highly conserved in all HIV genomes and thus provide potential targets for the selective inhibition of integration. The integrase-binding site located on the HIV-1 U5 LTR end contains two adjacent purine tracts on opposite strands, 5' . . . GGAAAATCTCT-3'/3'-CCTTTTAGAGA . . . 5', in parallel orientations. A single strand oligonucleotide 5'-GGTTTTTGTGT-3' was designed to associate with these tracts via its ability to form a continuous alternate strand DNA triplex. Under neutral pH and physiological temperature, the oligonucleotide, tagged with an intercalator chromophore oxazolopyridocarbazole, formed a stable triplex with the target DNA. The occurrence of this unusual triplex was demonstrated by both DNase I footprinting and electron microscopy. The triplex inhibits the two steps of the integrase-mediated reactions, namely, the endonucleolytic cleavage of the dinucleotide 5'-GT-3' from the 3' end of the integration substrate and the integration of the substrate into the heterologous target DNA. The midpoints for both inhibition reactions were observed at oligonucleotide concentrations of 50-100 nM. We believe that these results open new possibilities for the specific targeting of viral DNA LTR ends with the view of inhibiting integration under physiological conditions.

a. Alternate strand DNA triple helix-mediated inhibition of HIV-1 U5 long terminal repeat integration in vitro. **Bouziane M**, Cherny DI, Mouscadet JF, Auclair C. J Biol Chem. 1996 Apr 26;271(17):10359-64.
b. A molecular mechanics and dynamics study of alternate triple-helices involving the integrase-binding site of the HIV-1 virus and oligonucleotides having a 3'-3' internucleotide junction. Ouali M, **Bouziane M**, Ketterlé C, Gabarro-Arpa J, Auclair C, Le Bret M. J Biomol Struct Dyn. 1996 Apr;13(5):835-53.

c. A synthetic peptide from the human immunodeficiency virus type-1 integrase exhibits coiled-coil properties and interferes with the in vitro integration activity of the enzyme. Correlated biochemical and spectroscopic results., Sourgen F, Maroun RG, Frère V, **Bouziane M**, Auclair C, Troalen F, Fermandjian S., Eur J Biochem. 1996 Sep 15;240(3):765-73.
d. molecular mechanics and dynamics study of alternate triple-helices involving the integrase-binding site of the HIV-1 virus and oligonucleotides having a 3'-3' internucleotide junction. Ouali M, **Bouziane M**, Ketterlé C, Gabarro-Arpa J, Auclair C, Le Bret M. J Biomol Struct Dyn. 1996 Apr;13(5):835-53.

2. Repair of DNA alkylation damage.

Alkylation damage of DNA is one of the major types of insults which cells must repair to remain viable. One way alkylation damaged ring nitrogens are repaired is via the Base Excision Repair (BER) pathway. Examination of mutants in both BER and Nucleotide Excision Repair show that there is actually an overlap of repair by these two pathways for the removal of cytotoxic lesions in Escherichia coli. The enzymes removing damaged bases in the first step in the BER pathway are DNA glycosylases. The coding sequences for a number of methylpurine-DNA glycosylases (MPG proteins) were cloned, and a comparison of the amino-acid sequences shows that there are some similarities between these proteins, but nonetheless, compared to other DNA glycosylases, MPG proteins are more divergent. MPG proteins have been purified to homogeneity and used to identify their substrates ranging from methylating agents to deamination products to oxidatively damaged bases. The ligation-mediated polymerase chain reaction has been used to study the formation of alkylation damage, and its repair in mammalian cells. We have studied DNA damage in the PGK1 gene for a series of DNA alkylating agents including N-methyl-N'-nitro-N-nitrosoguanidine, Mechlorethamine, and Chlorambucil and shown that the damage observed in the PGK1 (phosphoglycerate kinase 1) gene depends on the alkylating agent used. This report reviews the literature on the MPG proteins, DNA glycosylases removing 3-methyladenine, and the use of these enzymes to detect DNA damage at the nucleotide level.

a. Interaction of the recombinant human methylpurine-DNA glycosylase (MPG protein) with oligodeoxyribonucleotides containing either hypoxanthine or abasic sites., **Bouziane M**, Miao F, O'Connor TR, Nucleic Acids Res. 1998 Sep 1;26(17):4034-41.

b. Promoter structure and cell cycle dependent expression of the human methylpurine-DNA glycosylase gene. **Bouziane M**, Miao F, Bates SE, Somsouk L, Sang BC, Denissenko M, O'Connor TR., Mutat Res. 2000 Sep 15;461:15-29.
c. Repair of Repair DNA alkylation damage**. Bouziane M**, Miao F, Ye N, Holmquist G, Chyzak G, O'Connor TR., Acta Biochim Pol. 1998;45(1):191-202.
d. 3-Methyladenine-DNA glycosylase (MPG protein) interacts with human RAD23 proteins. Miao F, **Bouziane M**, Dammann R, Masutani C, Hanaoka F, Pfeifer G, O'Connor TR., J Biol Chem. 2000 Sep 15;275:28433-8.

3. Sequence specific cleavage of DNA by a netropsin-flavin hybrid molecule.

In an attempt to obtain sequence specific DNA-cleaving molecules, I have synthesized a series of hybrid minor groove binders composed of a photoactiveable isoalloxazine (flavin) chromophore linked through a polymethylenic chain to a bis-pyrrolecarboxamide moiety related to netropsin. Like netropsin, the hybrid derivatives preferentially bind to A+T-rich sequences. Activation of the flavin chromophore by visible light results in the appearance of single strand breaks in the vicinity of the DNA binding site. I have further investigated the cleavage affinity properties of one of these compounds referred to as netropsin-flavin (Net-Fla) and considered as representative of the series. Net-Fla cleaves only one strand at a specific locus downstream of 5'-AAAT-3', upstream of 5'-TAAA-3' and on either side of a 5'-AAAA-3' sequence. Net-Fla cleaves both strands downstream to 5'-AATT-3'. This makes the properties of Net-Fla similar to that of a restriction endonuclease and provides additional insight into establishing the rules for the readout of B-DNA helix by non-nucleotidic compounds. Using molecular modeling, we show that Net-Fla binds to an asymmetric site in one orientation. The values of the energetic minima lie in the same order as expected from the cleavage patterns, which suggests that the oriented cleavage is a consequence of a sequence-oriented binding of Net-Fla in the DNA minor groove.

a. Sequence-directed single strand cleavage of DNA by a netropsin-flavin hybrid molecule. **Bouziane M**, Ketterlé C, Helissey P, Herfeld P, Le Bret M, Giorgi-Renault S, Auclair C. Biochemistry. 1995 Oct 31;34(43):14051-8.

b. Binding of Net-Fla, a netropsin-flavin hybrid molecule, to DNA: molecular mechanics and dynamics studies in vacuo and in water solution. Ketterlé C, Gabarro-Arpa J, Ouali M, Bouziane M, Auclair C, Helissey P, Giorgi-Renault S, Le Bret M., J Biomol Struct Dyn. 1996 Jun;13(6):963-77.

4. Overexpression of the cytokeratin 18 gene in tumorigenic clones relative to that in nontumorigenic clones of a human carcinoma cell line.

I have already reported that tumorigenic cells overexpress the cytokeratin 18 (K18) gene in comparison with nontumorigenic cells and that this difference is mainly due to a transcriptional regulation. We now report that a 2,532-bp cloned human K18 gene promoter drives the differential expression of a reporter gene in a transient assay. A 62-bp minimal K18 promoter (TATA box and initiation site) has a low but differential activity. Analysis of deletion and substitution mutants as well as hybrid SV40-K18 promoters and reconstructed K18 promoters indicated that an important element for the activity of the K18 promoter is a high-affinity binding site for transcription factor Sp1 located just upstream of the TATA box. This Sp1 binding element, as well as the intron 1 enhancer element, stimulates the basal activity of the minimal promoter through mechanisms that maintain the differential activity. Gel shift assays and the use of an anti-Sp1 antibody have shown that both tumorigenic and nontumorigenic SW613-S cells contain three factors able to bind to the Sp1 binding element site and that one of them is Sp1. A hybrid GAL4-Sp1 protein transactivated to comparable extents in tumorigenic and nontumorigenic cells a reconstructed K18 promoter containing GAL4 binding sites and therefore without altering its differential behavior. These results indicate that the Sp1 transcription factor is involved in the overexpression of the K18 gene in tumorigenic SW613-S cells through its interaction with a component of the basal transcription machinery.

a. An Sp1 binding site and the minimal promoter contribute to overexpression of the cytokeratin 18 gene in tumorigenic clones relative to that in nontumorigenic clones of a human carcinoma cell line. Gunther M, Frebourg T, Laithier, **Bouziane M**, Lavialle C, Brison Laboratoire de Génétique Oncologique, URA 1967 CNRS, Institut Gustave Roussy, Villejuif, France. Mol Cell Biol. 1995 May;15(5):2490-9.
b. Role of Fas and granule exocytosis pathways in tumor-infiltrating T lymphocyte- induced, apoptosis of autologous human lung-carcinoma cells. Dorothee G, Ameyar M, Bettaieb A, Vergnon I, Echchakir H, **Bouziane M**, Chouaib S, Mami-Chouaib F., Int J Cancer. 2001 Mar 15;91(6):772-7.

5. Efficient repair of cyclobutane pyrimidine dimers at mutational hot spots is restored in complemented Xeroderma pigmentosum

Xeroderma pigmentosum (XP) and trichothiodystrophy (TTD) are rare heritable diseases. Patients suffering from XP and 50% of TTD afflicted individuals are photosensitive and have a high susceptibility to develop skin tumors. One solution to alleviating symptoms of these diseases is to express the deficient cDNAs in patient cells as a form of gene therapy. XPC and TTD/XPD cell lines were complemented using retroviral transfer. Expressed wild-type XPC or XPD cDNAs in these cells restored the survival to UVC radiation to wild-type levels in the respective complementation groups. Although complemented XP cell lines have been studied for years, data on cyclobutane pyrimidine dimer (CPD) repair in these cells at different levels are sparse. We demonstrate that CPD repair is faster in the complemented lines at the global, gene, strand specific, and nucleotide specific levels than in the original lines. In both XPC and TTD/XPD complemented lines, CPD repair on the non-transcribed strand is faster than that for the MRC5SV line. However, global repair in the complemented cell lines and MRC5SV is still slower than in normal human fibroblasts. Despite the slower global repair rate, in the complemented XPC and TTD/XPD cells, almost all of the CPDs at "hotspots" for mutation in the P53 tumor database are repaired as rapidly as in normal human fibroblasts. Such evaluation of repair at nucleotide resolution in complemented nucleotide excision

repair deficient cells presents a crucial way to determine the efficient re-establishment of function needed for successful gene therapy, even when full repair capacity is not restored.

a. Efficient repair of cyclobutane pyrimidine dimers at mutational hot spots is restored in complemented Xeroderma pigmentosum group C and trichothiodystrophy/xeroderma pigmentosum group D cells., Zhou NY, Bates SE, **Bouziane M**, Stary A, Sarasin A, O'Connor TR., J Mol Biol. 2003 Sep 12;332(2):337-51.
b. Crosstalk between extrinsic and intrinsic cell death pathways in pancreatic cancer: synergistic action of estrogen metabolite and ligands of death receptor family. Basu A, Castle VP, Bouziane M, Bhalla K, Haldar S., Cancer Res. 2006 Apr 15;66(8):4309-18.
c. Regulation of autoimmune arthritis by the pro-inflammatory cytokine interferon- gamma. Kim EY, Chi HH, Bouziane M, Gaur A, Moudgil KD., Clin Immunol. 2008 Apr;127(1):98-106. Epub 2008 Feb 13.
d. Age-dependent effects of nongenotoxic hepatocarcinogens on liver apoptosis in vivo. Youssef JA, Bouziane M, Badr MZ., Mech Ageing Dev. 2003 Mar;124(3):333-40.

Complete List of Published Work in My Bibliography:
(https://www.ncbi.nlm.nih.gov/pubmed/?term=bouziane+m)

D. Research Support

1. **Ongoing:**
2. R41 AI138764-01 (**Bouziane, Co-PI**). A Novel Self-Assembling Protein Nanoparticles-Based Genital Herpes Vaccine. NIH/NIAID Period: **07/01/18 - 06/30/2019.**

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Burkhard, Peter

eRA COMMONS USER NAME (credential, e.g., agency login): PETERBURKHARD

POSITION TITLE: CEO

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Biozentrum, University of Basel, Basel, Switzerland	Diploma	1992	Biochemistry
Sandoz Pharma AG, Basel, Switzerland	PhD	1995	Biophysics
Biozentrum, University of Basel, Basel, Switzerland	Postdoc	1998	Structural Biology
Biozentrum, University of Basel, Basel, Switzerland	Habilitation	2001	Structural Biology

A. Personal Statement

My experience and qualifications make me particularly well-suited for the role as co-PI in this HSV nanoparticle vaccine project. The nanoparticles have been invented by me about fifteen years ago. Ever since I have continuously and with great enthusiasm further developed the nanoparticles to make them suitable as a platform for vaccine design. I have analyzed their biophysical and immunological properties in great detail resulting in the production of five vaccine prototypes for four different infectious diseases that all are almost completely protective in animal challenge models. This work resulted in five different patents / patent applications of SAPNs for vaccine design (US8575110, US8546337, EP2766386A1, EP2766386A1, EP17157687.9). I have also advanced our most developed malaria SAPN vaccine up to the stage of clinical trials phase I/IIa, which is planned to be finished in summer (2018). Furthermore, I have founded the company Alpha-O Peptides more than a decade ago. Developing vaccines is the core business of this company. For all those reasons I think that I am perfectly qualified to be the lead PI at Alpha-O Peptides in this HSV vaccine project. I strive to develop the HSV vaccine to possibly also bring it into clinical trials as quickly as possible. My personal background covering everything from nano-biotechnology to immunology and including all aspects that are important for vaccine design makes me perfectly suited to direct the research of this proposal. I will do this in close collaboration with the other PIs Dr. BenMohamed and Dr. Bouziane from the University of California Irvine and Sunomix Biosciences. The following are my most important patents / patent applications.

a) US8575110 (2004). “Peptidic Nanoparticles as Drug Delivery and Antigen Display Systems” **P. Burkhard**
b) US 8546337 (2008). “Self-assembling peptide nanoparticles useful as vaccines” **P. Burkhard**
c) WO2015104352 (2014). “Flagellin-containing protein nanoparticles as a vaccine platform” by S.K. Raman, S.M. Paulillo, M. Piazza, C. Kulangara, C. Mittelholzer, and **P. Burkhard**
d) EP17157687.9 (2017). “Self-assembling protein nanoparticles encapsulating immunostimulatory nucleid acids” by S.K. Raman, S.M. Paulillo, C. Kulangara, M. Piazza and **P. Burkhard**

B. Positions and Honors

- 1995 - 1998 Postdoctoral Position at the Biozentrum, University of Basel, CH
- 1998 - 2004 Group leader at the Biozentrum, University of Basel, CH
- 2001 Habilitation, University of Basel, CH
- 2003 Founder of Alpha-O Peptides, AG, Riehen, CH
- 2004 - 2013 Associate Professor, University of Connecticut, CT, USA
- 2013 - 2015 Full Professor, University of Connecticut, CT, USA
- 2015 - 2016 Research Professor, University of Connecticut, CT, USA
- 2003 - CEO of Alpha-O Peptides, AG, Riehen, CH

- 2005 Senior Founding Member of the American Academy of Nanomedicine
- 2006 Fellow of the American Academy of Nanomedicine
- 2010 Tenured faculty position at the University of Connecticut
- 2011 - Editor of the Journal of Nanobiotechnology
- 2011 Director's Award for Faculty Excellence, Polymer Program, University of Connecticut
- 2012 - Editor of Current Bionanotechnology

C. Contribution to Science

1. Protein Structural Analysis for Structure Based Design

DOPA decarboxylase (DDC) is responsible for the synthesis of the key neurotransmitters dopamine and serotonin via decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan, respectively. DDC has been implicated in a number of clinic disorders, including Parkinson's disease and hypertension. Peripheral inhibitors of DDC are currently used to treat these diseases. We have solved the X-ray crystal structures of ligand-free DDC and its complex with the anti-Parkinson drug carbiDOPA. The inhibitor is bound to the enzyme by forming a hydrazone linkage with the cofactor, and its catechol ring is deeply buried in the active site cleft. These structures provide the molecular basis for the development of new inhibitors of DDC with better pharmacological characteristics. P. Burkhard et al. (2001) Nature Struct Biol, 8 (11), 963 – 967).

Publication of these DDC structures prompted Rebecca Craven to write the following comments in the Highlights section in Nature Reviews Neuroscience (2002) 2 (12), 855: *The treatment of patients with Parkinson's disease could be greatly improved by the design of more effective inhibitors of this enzyme. This prospect seems increasingly likely, as Burkhard et al. report the crystal structures of ligand-free DCC, and its complex with carbiDOPA. Importantly, on the basis of these structures, the authors were able to suggest ways in which the binding of inhibitors of DCC might be improved. The use of more-potent inhibitors of DCC would allow smaller amounts L-DOPA to be used in alleviating the symptoms of Parkinson's disease; the crystal structures reported by Burkhard et al. offer a way forward in the design of such treatments.*

e) **Burkhard, P.**, Dominici, P., Borri-Voltattorni, C., Jansonius, J.N., and Malashkevich, V.N. Structural insight into Parkinson's disease treatment gained from drug-inhibited DOPA decarboxylase. *Nature Struct Biol*. 2001 Nov;8(11):963-967. PMID: 11685243

f) Meier, M., Janosik, M., Kery, V., Kraus, J. and **Burkhard, P**. Structure of human cystathionine beta-synthase: a unique pyridoxal 5'-phosphate-dependent heme protein. *EMBO J*. 2001 Aug 1;20(15):3910-3916. PMCID: PMC149156

g) Stetefeld, J., Jenny, M., and **Burkhard, P**. Intersubunit signaling in glutamate-1-semialdehyde-aminomutase. *Proc Natl Acad Sci U S A*. 2006 Sep 12;103(37):13688-13693. PMCID: PMC1564225

h) **Burkhard, P**., Rao, G.S., Hohenester, E., Schnackerz, K.D., Cook, P.F. & Jansonius, J.N. Three-dimensional Structure of *O*-acetylserine Sulfhydrylase from Salmonella typhimurium. *J Mol Biol*. 1998;283(1):121-133. PMID: 9761678

2. Structural Design and Analysis of Coiled-coil Proteins

The parallel two-stranded α-helical coiled coil is the most frequently encountered subunit-oligomerization motif in proteins. The simplicity and regularity of this motif have made it an attractive system to explore some of the fundamental principles of protein folding and stability and to test the principles of de novo design. We have solved the X-ray crystal structure of the 18-heptad-repeat α-helical coiled-coil domain of the actin-bundling protein cortexillin I from Dictyostelium discoideum and shown that it is a tightly packed parallel two-stranded α-helical coiled coil. It harbors a distinct 14-residue sequence motif that is essential for coiled-coil formation, and is a prerequisite for the assembly of cortexillin I. The knowledge gained from the structure can be used in the de novo design of α-helical coiled coils for applications such as two-stage drug targeting and delivery systems, and in the design of coiled coils as templates for combinatorial helical libraries in drug discovery and as synthetic carrier molecules. (P. Burkhard et al. (2000). *Structure*, **8**, 223-230.)

Presentation of this structure at the American Crystallographic Association Annual Meeting 1999 in Washington triggered the following Editorial Reprise in *Nature Struct. Biol*., 5, (1998), 762 by Guy Riddihough. *Perhaps the most apposite example was provided by P. Burkhard who reported on the structure determination of the 190 Å long α-helical, two-stranded, right-handed coiled-coil rod domain from cortexillin I. This is the longest structure of a coiled coil reported to date, soundly beating the 39-residue long cFos-cJun bZIP leucine zipper. The rod domain includes a 13-residue 'trigger site' that has been shown to be necessary for coiled coil assembly and, indeed, has been characterized as an autonomous folding unit, suggesting that this is a general feature of coiled coil assembly.*

a) Strelkov, S., Herrmann, H., Geisler, N., Zimbelmann, R., Aebi, U. and **Burkhard, P**. Conserved segments 1A and 2B of the intermediate filament dimer: their atomic structures and role in filament assembly. *EMBO J*. 2002 Mar 15;21(6):1255-1266. PMCID: PMC125921

b) Strelkov, S.V., and **Burkhard, P**. Analysis of alpha-helical coiled coils with the program TWISTER reveals a structural mechanism for stutter compensation. *J Struct Biol*. 2002 Jan-Feb;137(1-2):54-64. PMID: 12064933

c) **Burkhard, P**., Kammerer, R.A., Steinmetz, M.O., Bourenkov, G.P. and Aebi, U. The coiled-coil trigger site of the rod domain of cortexillin I unveils a distinct network of inter- and intra-helical salt-bridges. *Structure*. 2000 Mar 15;8(3):223-230. PMID: 10745004

d) **Burkhard, P**., Meier, M. and Lustig, A. Design of a minimal protein oligomerization domain by a structural approach. *Protein Science*. 2000 Dec;9(12):2294-2301. PMCID: PMC2144530

3. Structural Design of Self-Assembling Protein Nanoparticles (SAPNs)

Artificial particulate systems such as polymeric beads and liposomes are being applied in drug delivery, drug targeting, antigen display, vaccination, and other technologies. We have used computer modeling to design a novel type of self-assembling protein nanoparticles (SAPNs) composed of proteins as building blocks. We describe the structure-based design of a novel type of nanoparticles with regular polyhedral symmetry and a diameter of about 16 nm, which self-assembles from single protein chains. Each protein chain is composed of two coiled coil oligomerization domains with different oligomerization states joined by a short linker segment. In aqueous solution the proteins form nanoparticles of about 20 nm diameter. Such protein nanoparticles are ideally suited for medical applications such as drug targeting and drug delivery systems, as imaging devices, or they may be used for repetitive antigen display.

a) Raman, S.K., Machaidze, G., Lustig, A., Aebi, U. and **Burkhard, P**. Structure-based design of peptides that self-assemble into regular polyhedral nanoparticles. *Nanomedicine*. 2006 Jun;2(2):95-102. PMID: 17292121

b) Pimentel T.A., Yan Z, Jeffers S.A., Holmes K.V., Hodges R.S. and **Burkhard P**. Peptide nanoparticles as novel immunogens: design and analysis of a prototypic severe acute respiratory syndrome vaccine. *Chemical Biology and Drug Design*. 2009 Jan;73(1):53-61. PMCID: PMC2756483

c) Yang Y., Ringler P., Mueller S.A. and **Burkhard P**. Optimizing the refolding conditions of self-assembling polypeptide nanoparticles that serve as repetitive antigen display systems. *J Struct Biol.* 2012 Jan;177(1):168-176. PMID: 22115997

d) Indelicato G., Wahome N., Ringler P., Müller S.A., Nieh M., **Burkhard P** and Twarock R. Principles Governing the Self-Assembly of Coiled-Coil Protein Nanoparticles. *Biophys J*. 2016 Feb 2;110(3):646-660. PMCID: PMC4744166

4. Vaccine design using SAPNs

Using the SAPNs as a platform for vaccine design, I have demonstrated that the SAPNs can be used as a general platform for vaccine design. I have five different patents / patent applications dealing with the use of SAPNs for vaccine design (US8575110, US8546337, EP2766386A1, EP2766386A1, EP17157687.9). In the research labs of Alpha-O Peptides we have engineered five vaccine prototypes for four different infectious diseases that all are almost completely protective in animal challenge models. The clinical trials phase I/IIa of the most advanced vaccine (malaria) is currently planned to be finished next summer (2018). The five prototypes are: Malaria vaccine, HPV vaccine (L2-based), universal flu vaccine (M2e- and Helix C-based), seasonal flu vaccine (HA-based), toxoplasmosis vaccine. All of those prototypes are bacterially expressed, most of them are composed of one single protein chain. So, they can be produced very cheaply and rapidly. These five prototype vaccines show that the SAPN technology is indeed a platform technology that can be quickly adapted to pretty much any infectious disease (Ebola, Zika, Chikungunya, etc.). Furthermore, the SAPN technology can be used to engineer therapeutic vaccines for cancer, Alzheimer, addictions, obesity and many more.

a) Kaba, S.A., McCoy, M.E., Doll T.A., Brando C., Guo Q., Dasgupta D., Yang Y., Mittelholzer C., Spaccapelo R., Crisanti A., **Burkhard P**. and Lanar D.E. Protective Antibody and CD8+ T-Cell Responses to the Plasmodium falciparum Circumsporozoite Protein Induced by a Nanoparticle Vaccine. *PLoS One*. 2012;7(10):e48304. PMCID: PMC3483151
b) Kaba S.A., Brando C., Guo Q., Mittelholzer C., Raman S.K., Tropel D., Aebi U., **Burkhard P**. and Lanar D.E. A nonadjuvanted polypeptide nanoparticle vaccine confers long-lasting protection against rodent malaria. *J Immunol*. 2009 Dec 1;183(11):7268-7277. PMCID: PMC4528972
c) El-Bissati K., Zhou Y., Dasgupta D., Cobb D., Dubey J.P. and **Burkhard P**., Lanar D.E., McLeod R. Effectiveness of a novel immunogenic nanoparticle platform for Toxoplasma peptide vaccine in HLA transgenic mice. *Vaccine*. 2014 May 30;32(26):3243-3248. PMCID: PMC4084734
d) Karch CP, Li J, Kulangara C, Paulillo SM, Raman SK, Emadi S, Tan A, Helal ZH, Fan Q, Khan MI, Burkhard P. Vaccination with self-adjuvanted protein nanoparticles provides protection against lethal influenza challenge. *Nanomedicine*. 2017 Jan;13(1):241-251. doi: 10.1016/j.nano.2016.08.030.

Complete List of Published Work in NCBI

https://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/42368162/

D. Research Support

Ongoing Research Support

Development of novel IBV-nanoparticle based vaccine, its immunogenicity and protection studies in chickens

Role: Co-PI Duration: 05/15 - 04/18 Funding agency: USDA-NIFA
Overall goal: To design protein nanoparticles as subunit vaccine against IBV.
Responsibilities: To direct the research in the Burkhard lab at UConn and coordinate with the research group of the PI M. Khan at the University of Connecticut.

GMP Production and Clinical Trial of a Self-Assembling Protein Nanoparticle and Toll-Like Receptor Liposomal MPL Adjuvanted Malaria Vaccine

Role: Co-PI Duration: 07/15 - 06/17 Funding agency: CDRMP
Overall goal: To test a malaria vaccine based on self-assembling protein nanoparticles in clinical trials.
Responsibilities: To consult on the bio-production and vaccination protocols for the self-assembling protein nanoparticles developed at Alpha-O Peptides AG.

Completed Research Support

Malaria Vaccine Based on Self-Assembling Polypeptide Nanoparticles (SAPN)

Role: PI Duration: 08/09 - 07/13 Funding agency: NIH-NIAID
Overall goal: This R01 proposal has the goal to design peptide nanoparticles as subunit vaccine against malaria.
Responsibilities: To direct the research at UConn and coordinate with the research group at WRAIR.

Atomic structure and assembly of Intermediate Filaments

Role PI Duration: 05/11 - 12/16 Funding agency: NIH-NIGMS

Overall goal: The goal of this PPG-project is to investigate the structural and biophysical properties of the intermediate filament protein vimentin

Responsibilities: To direct the research in the Burkhard lab at UConn and coordinate with the research group at Harvard, Northwestern and UPenn.

A peptide nanoparticle nicotine vaccine

Role PI Duration: 09/11 - 12/16 Funding agency: NIH-NIDA

Overall goal: This DP1 award aims at the development of a peptide nanoparticle nicotine vaccine and advance it through clinical trials phase I.

Responsibilities: To direct the whole project at UConn (protein design) at Alpha-O Peptides in Riehen (biophysical analysis), Switzerland and the Kantonsspital St. Gallen, Switzerland (clinical trials).

Peptide Nanoparticles as Novel Immunogens: Design and Analysis of Avian Influenza Vaccine

Role PI Duration: 12/11 - 11/16 Funding agency: USDA-NIFA

Overall goal: To design peptide nanoparticles as subunit vaccine against malaria.

Responsibilities: To direct the research in the Burkhard lab at UConn and coordinate with the research group of Dr. Khan (UConn - PI) and Gelb (University of Maryland).

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OMB Number: 0925-0001

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[redacted]

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12758901 Funding Opportunity Number: PA-18-489 . Received Date: 2018-12-10T19:07:05.000-05:00

PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Budget Period: 1

Start Date: 07/01/2019 End Date: 06/30/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	125,000.00
Consortium Indirect (F&A)	17,500.00
Total Direct Costs*	142,500.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	54.50	87,500.00	47,688.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Robert W. Lee, (415) 437-7820

Indirect (F&A) Rate Agreement Date: 04/27/2011

Total Indirect (F&A) Costs: 47,688.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 190,188.00

PHS 398 Modular Budget

Budget Period: 2			
Start Date: 07/01/2020 End Date: 06/30/2021			
A. Direct Costs			Funds Requested ($)
	Direct Cost less Consortium Indirect (F&A)*		150,000.00
	Consortium Indirect (F&A)		21,000.00
	Total Direct Costs*		171,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	54.50	75,000.00	40,875.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Robert W. Lee, (415) 437-7820

Indirect (F&A) Rate Agreement Date: 04/27/2011

Total Indirect (F&A) Costs: 40,875.00

C. Total Direct and Indirect (F&A) Costs (A + B)	Funds Requested ($)	211,875.00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	275,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	38,500.00
Section A, Total Direct Costs for Entire Project Period ($)	313,500.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	88,563.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	402,063.00

2. Budget Justifications

Personnel Justification	PersonneJustification1010649552.pdf
Consortium Justification	Consortium_Justification1010649510.pdf
Additional Narrative Justification	Additional_Narrative_Justification1010649266.pdf

PERSONNEL JUSTIFICATION

University of California, Irvine

PERSONNEL:

Lbachir BenMohamed, Ph. D. **Principal Investigator** **1.2 Calendar Months**
Dr. BenMohamed (Professor/Director of Cellular and Molecular Immunology Laboratory) is requesting 1.2 calendar months time and effort. He is a faculty member at the Gavin Herbert Eye Institute and has joint appointments at the Institute for Immunology of the University of California Irvine (UCI). He is an immunologist with expertise in herpes simplex infection and immunity in both animal models and in humans. He has a 20-year background in cellular and molecular immunology, and is a leading researcher in ocular herpes vaccine and immunotherapy. For the last 15 years, the PI has accumulated extensive research experience in the field of herpes immunity and immunopathology from The Pasteur Institute (Paris), The City of Hope National Medical Center, Cedars Sinai Medical Center and more recently in the Laboratory of Cellular and Molecular Immunology at UCI. Dr. BenMohamed will be directly involved in immunization and studying the immunogenicity and protective efficacy of SAPN-based for vaccine against ocular herpes in [redacted] 6255 [redacted]

Nisha Dhanushkodi, Ph. D **Postdoctoral Fellow** **2.4 CM/Yr1 & 7.8 CM/Yr2**
[redacted] 6255 [redacted]

Christine McLaren, Ph. D. **Collaborator** **0.24 Calendar Months**
We are requesting 0.24 calendar month time and effort for Dr. McLaren, the Director of Biostatistics at the Department of Epidemiology (UC, Irvine). Dr. McLaren is a professor of Epidemiology and Bio-statistics at UC Irvine. She will help with the statistical analysis as described in this application. Dr. BenMohamed and Dr. McLaren have been collaborating for the last 6- years on many ongoing herpes immunology projects.

Peter Burkhhard, Ph. D. **Collaborator** **<0.1 Calendar Months**
For the design, construction an bio-production of SAPNs nanoparticles for this proposal, there is Dr. Burkhhard. He has over 20 years in antigen delivery systems, including the nanoparticles described in this application. He will be available on an as needed basis.

CONSORTIUM JUSTIFICATION

Sunomix Therapeutics, San Diego, CA

Year 1: $62,500 Direct Cost, $17,500 indirect cost; $80,000 total cost
Year 2: $75,000 Direct Cost, $21,000 indirect cost; $96,000 total cost

Mohammed Bouziane, Ph. D CO-Principal Investigator 1.5 Calendar Months

Dr Bouziane has extensive experience in leading multi-disciplinary groups from Discovery, R&D to preclinical, and early clinical programs. Strong and broad scientific expertise covering multiple areas of genomics, proteomics, mutagenesis, nanoparticules SAPNs, diagnostics, vaccines and cell therapies for the treatment of infectious diseases, cancer and immunotherapies.

Dr Bouziane is the Co-principal investigator on this this HSV nanoparticle vaccine project and responsible for its conception and in coordinating the collaboration. Alpha-O Peptides owns valuable technology, and intellectual property and is working exclusively with Sunomix Therapeutics for the technology transfer and the development of an effective SAPNs-based herpes vaccine.

Sunomix therapeutics is using the approved and validated bio-production protocol and will be responsible to deliver the 18 SAPNs to Dr Lbachir Benmohamed Lab at UCI, California, to be used for the Herpes vaccine grant. As Sunomix Therapeutics CEO, Dr Bouziane will be directly involved in the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for HSV vaccine. [redacted] in Dr Benmohamed lab will be produced at Sunomix Therapeutics. All the nanoparticles will contain [redacted] using SAPNs produced by Sunomix Therapeutics shows very promising results.
Dr Bouziane will also assist the PI with the in vivo work and data analysis as described in this application. He will supervise a postdoctoral fellow. They will meet weekly to discuss results of SAPNs bioproduction.

Research Scientist 6 Calendar Months

We are requesting 6 calendar months' time and effort for this research scientist fellow. He will be responsible for the bioproduction of SAPNs under Dr Bouziane supervision including: construction design, PCR, cloning, sequencing, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for HSV vaccine. [redacted]

Additional Narrative Justification

Note: The Additional Narrative Justification is not needed in applications to FOAs with direct cost limits that do not spread evenly across budget periods (e.g., R21 FOAs that allow $275,000 in direct costs over two years).

Please see guideline on the link below.
https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.320-phs-398-modular-budget-form.htm

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SpecificAims1010649541.pdf
3. Research Strategy*	ResearchStrategy1010649542.pdf
4. Progress Report Publication List	
Other Research Plan Section	
[redacted]	
6. Select Agent Research	BiohazardsHSV_11010649544.pdf
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	ConsortiumContractual_Arrangements1010649545.pdf
9. Letters of Support	LettersofSupport1010649546.pdf
10. Resource Sharing Plan(s)	ResourceSharingPlan1010649547.pdf
11. Authentication of Key Biological and/or Chemical Resources	BiologicalandChemicalResources1010649553.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT12758901 Funding Opportunity Number: PA-18-489. Received Date: 2018-12-10T19:07:05.000-05:00

Specific Aims:

A staggering number of individuals—over 3 billion worldwide—are currently infected with herpes simplex virus type 1 (HSV-1)[1-7]. Recurrent herpetic stromal keratitis (rHSK), due to reactivation of HSV-1 from latency, can cause corneal blindness.

To test these hypotheses, we propose two synergistic Specific Aims:

Aim 1: Test the hypotheses that therapeutic immunization of HSV-1-infected HLA double transgenic 6255 with SAPNs-based herpes vaccines delivering

6255

Aim 2: Test the hypotheses that therapeutic immunization of latently infected HLA double transgenic 6255 with SAPNs-based herpes vaccines incorporating

6255

Outcome:

6255

[redacted]

APPROACH

Sex variables: This proposal will use an established female mouse model of UV-B-induced recurrent ocular herpes. At least one experiment will be done directly comparing males and females infected ocularly. If we observe sex-dependent differences, these results will be the foundation for a future grant application.
Experimental rigor and reproducibility: We have the following mechanisms in place to ensure rigor and reproducibility. All experiments will include appropriate positive and negative controls [redacted]

[redacted]

Aim 1: **Test the hypotheses that therapeutic immunization of HSV-1 infected** [redacted] **with SAPNs-based herpes vaccines delivering** [redacted]

[redacted]

Experimental design: The immunogenicity and protective efficacy [redacted]

[redacted]

(**Experiment 1**): **Construction of SAPNs-based herpes vaccines** [redacted]

[redacted]

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[redacted]

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(**Experiment 4**)

vs. CD8+ T cells.

Expected results and interpretations: (**Experiment 1**)

Potential Pitfalls and **Alternative Approaches:** This application focuses on T cell vaccines; however, virus neutralizing antibodies (IgG and IgM) will also be analyzed[74, 80]. **Outcome**: A candidate HSV-1 "protective" VP16 and VP22-SAPN vaccine to be used in a future herpes vaccine clinical trial is expected.

Aim 2: Test the hypotheses that therapeutic immunization of latently infected HLA double transgenic 6255 **with**

Rationale: We previously found that immunization with a mixture of three HSV T cell peptide epitopes induced better T cell-dependent protection against herpes than any single peptide epitope alone [80]. This finding strongly suggests that multi-epitopes can induce a robust T cell-mediated protective immunity [74, 80]. This is

Experimental design: (**Experiment 1**)

immunological assays as described in **Experiments #2, #3,** and **#4** in Aim1 above. ***Statistics:*** Analyses will be performed as aforementioned in Aim 1.

Expected results and **interpretation**: We expect that the SAPNs-based vaccine incorporating a

In addition, the more epitopes that can be included without producing negative effects (such as interfering with each other), the more likely that the T cell responses will be broader.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

[redacted]


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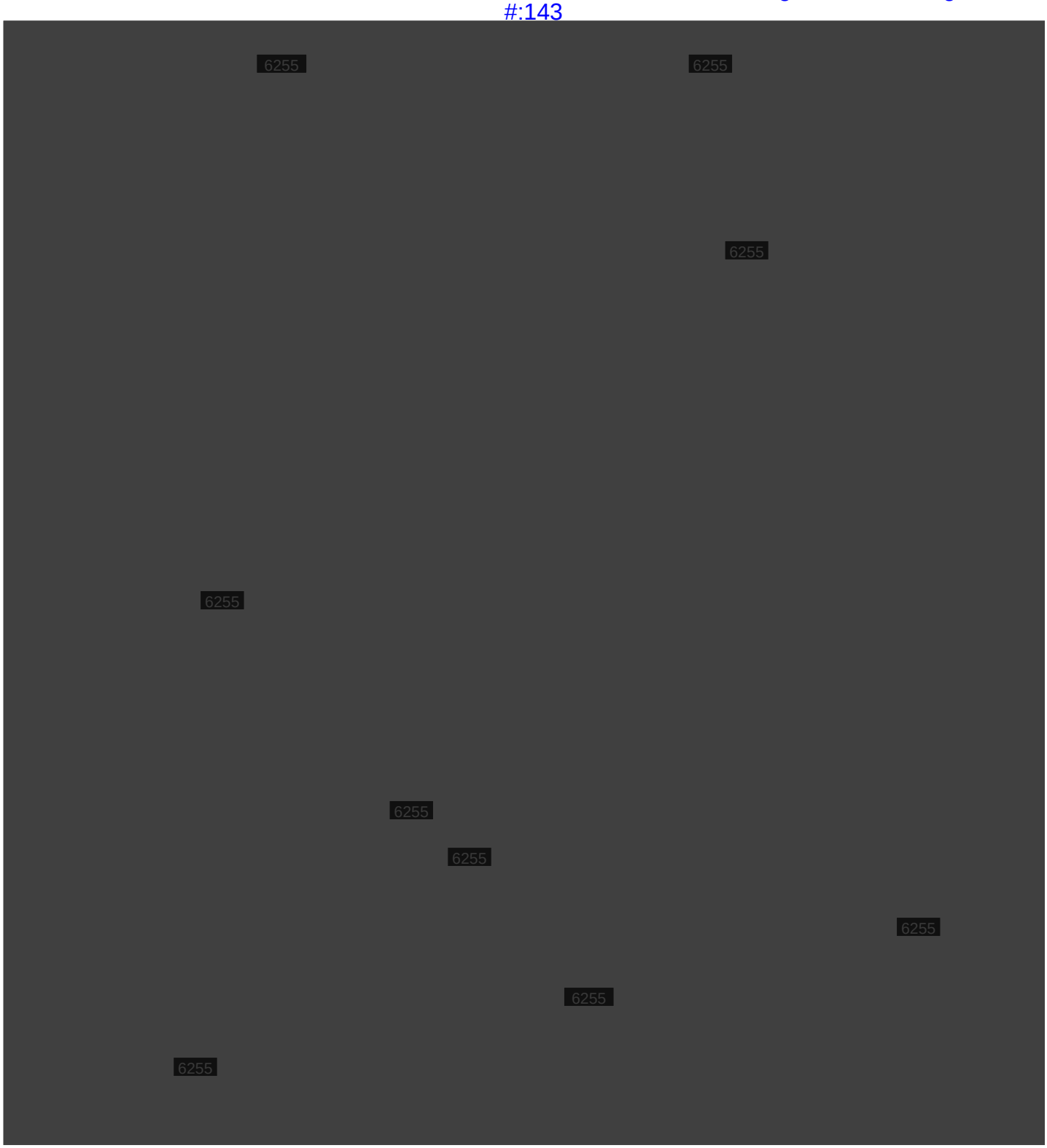
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BIOHAZARDS

Herpes simplex virus type 1 (HSV-1) is a biosafety level 2 (BSL-2) biohazard. All HSV-1 work done in the lab is performed using BSL-2 level precautions [redacted]

Laboratory environment and safety: Our laboratory is equipped for BSL-2 research and certified by UC Irvine Environmental Health and Safety and the IBC. HSV-1 is handled under biosafety level 2 conditions using BSL-2 rated biosafety cabinets.

As indicated in our IBC protocol, all virus work in the lab is done in a specific room (room 2337) within our main lab. This “virus” room is dedicated to HSV-1 work. For work involving infection of tissue culture cells with HSV-1, “clean” cells are brought to room 2337 from our clean tissue culture room (just a few yards away) and used in the BSL-2 certified Biosafety cabinets dedicated to virus work. Infected cell cultures are incubated in CO2 incubators in the same room. Personal Protective Equipment (PPE) for work in a Biosafety hood is labcoat and gloves. All surfaces are disinfected with 10% bleach after use. All HSV-1 liquid waste is incubated in a 10% solution (final concentration) of bleach for at least 30 minutes and then disposed of down a drain. Solid HSV-1 waste (plasticware) is disposed of as solid Medical Waste in properly labeled biohazard waste containers and collected by Environmental Health & Safety. The approved protocols are updated and renewed every year. Our lab is (as are all the labs in our building) subject to regular inspection by Environmental Health & Safety, IBC, and various state agencies, to assure compliance with all regulations regarding the use of biohazardous agents and infectious waste. All lab personnel have to take various biosafety courses, including “blood borne pathogens”, and receive specific HSV-1 safety training by a senior level individual in the lab, prior to working with HSV-1. The door to the lab as well as the specific HSV-1 room has a biohazard warning label indicating that HSV-1 is used inside.

Personnel training: Dr. BenMohamed (PI) has over 15 years experience utilizing HSV-1 in laboratory settings. All laboratory workers, including the PI, who work with animals or HSV-1 are required to take yearly specific training in laboratory safety at UCI including: (*i*) Wearing proper PPE (gloves, eye protection, labcoat etc...) when manipulating HSV-1 and HSV-1 infected tissues; (*ii*) proper virological techniques; (*iii*) the proper use and disposal of biohazardous agents and infectious waste into biohazardous waste containers; (*iv*) biohazard training; (*v*) Aerosol Transmissible Diseases (ATD) training; (*vi*) medical waste handling; and (*vii*) shipment of biohazardous materials. All new personnel are handed Standard Operating Procedures (SOP) and trained upon arrival and then on an annual basis. Research support services include training classes; seminars and wet labs are offered at UCI throughout the year.

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SCOPE OF WORK

Sunomix Therapeutics, Inc. San Diego, CA

Dr. Bouziane have extensive experience in leading multi-disciplinary groups from Discovery, R&D to preclinical, and early clinical programs. Strong and broad scientific expertise covering multiple areas of genomics, proteomics, mutagenesis, nanoparticles SAPNs, diagnostics, vaccines and cell therapies for the treatment of infectious diseases, cancer and immunotherapies.

He will serve Co-Investigator on t\\this HSV nanoparticle vaccine project and responsible for its conception and in coordinating the collaboration. Alpha-O Peptides owns valuable technology, and intellectual property and is working exclusively with Sunomix Therapeutics for the technology transfer and the development of an effective SAPNs-based herpes vaccine.

Sunomix therapeutics is using the approved and validated bio-production protocol and will be responsible to deliver the 18 SAPNs to Dr. BenMohamed Lab at UCI, California, to be used for the ocular herpes vaccine grant entitled "*PROTECTIVE IMMUNITY AGAINST RECURRENT OCULAR HERPES INDUCED WITH SELF-ASSEMBLING PROTEIN NANOPARTICLES*".

As Sunomix Therapeutics CEO, Dr. Bouziane will be directly involved in the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for HSV vaccine. At least eighteen prototype that contain different pairs of CD4 and CD8 human epitopes identified from herpes genome in Dr. BenMohamed lab will be produced at Sunomix Therapeutics. All the nanoparticles will contain CpG, the preliminary data using SAPNs produced by Sunomix Therapeutics shows very promising results.

Dr. Bouziane will also assist the PI with the in vivo work and data analysis as described in this application. He will supervise a postdoctoral fellow. They will meet weekly to discuss results of SAPNs bio production.

Sunomix Therapeutics

San Diego, December 8nd, 2018

Dear Dr. BenMohamed,

As a co-investigator on your new HSV nanoparticle-based ocular herpes therapeutic vaccine project entitled" *SELF-ASSEMBLING PROTEIN NANOPARTICLES-BASED OCULAR HERPES THERAPEUTIC VACCINE*", I will be responsible for the design and construction of the Self Assembling Protein Nanoparticles (SAPNs) you proposed in your new R21 grant proposal.

As a business partner, Sunomix Therapeutics, Inc. will provide you self-assembling protein nanoparticles (SAPNs) that will incorporate the whole VP16 and VP22 tegument protein (Aim 1) and your recently discovered human CD4 and $CD8^+$ T cell epitopes from HSV-1 VP16 and VP22 tegument protein (Aim 2).

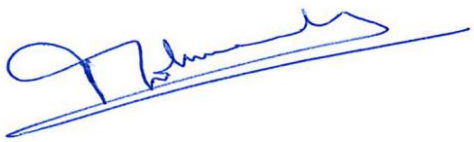
I will direct the design, development, cloning, protein expression, purification, refolding, TLR-activation and analysis of SAPN prototype to make them suitable as a platform for your ocular herpes therapeutic vaccine.

[redacted]

Developing vaccines is the core business of Sunomix Therapeutics. I believe that I am qualified to be the lead co-investigator in your HSV vaccine project.

Looking forwards to a successful collaboration

With Best Regards



Mohammed Bouziane, Ph. D.

CEO, Sunomix Therapeutics, Inc.

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Lbachir BenMohamed, PhD.
Professor & Director
Cellular & Molecular Immunology Laboratory
The Gavin Herbert Institute
Institute for Immunology
UC Irvine, School of Medicine
University of California Irvine

Riehen, December 10, 2018

Dear Dr. BenMohamed,

As you know, Alpha-O peptides owns valuable technology, intellectual property (US8575110, US8546337, US2014/0242104A1, EP3092245A1, and EP17157687.9) and proprietary information related to the design, construction, and bio-production of self-assembling protein nanoparticles (SAPNs). For this project of engineering ten pairs of CD4/CD8 T-cell epitopes into the SAPNs, Alpha-O Peptides has executed a separate written agreement to work exclusively with Sunomix Therapeutics and to transfer the technology for the bio-production of such SAPN-based HSV-vaccines from Alpha-O Peptides to Sunomix Therapeutics. Sunomix Therapeutics will use the validated bio-production protocol provided by Alpha-O to generate similar HSV-SAPN constructs. Sunomix has in the meantime acquired the know-how of the production of SAPN-based vaccines based on previous collaborations with Alpha-O Peptides.
Sunomix Therapeutics will be responsible to deliver at least 2 mg of pure SAPN-protein of the constructs to your laboratory at the University of California, Irvine, to be used for the immunization experiments of the project described in the ocular herpes vaccine grant proposal.

[redacted]

Yours sincerely,

Peter Burkhard

CEO, Alpha-O Peptides AG

UNIVERSITY OF CALIFORNIA, IRVINE

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

Please reply to:

DEPARTMENT OF EPIDMIOLOGY
UCI SCHOOL OF MEDICINE

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December 4th, 2018

Dr. Lbachir BenMohamed, PhD.
Professor & Director
Cellular & Molecular Immunology Laboratory
The Gavin S. Herbert Eye Institute & Center for Immunology
UC Irvine, School of Medicine
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843 Health Sciences Rd
Irvine, CA 92697-4390

Dear Lbachir,

I am writing to confirm that I will collaborate on your new R21 grant proposal entitled *"PROTECTIVE IMMUNITY AGAINST RECURRENT OCULAR HERPES INDUCED WITH SELF-ASSEMBLING PROTEIN NANOPARTICLES*" to be submitted to the National Eye Institute.

I look forward to providing expertise in biostatistics with regard to study design, monitoring, and data analysis protective efficacy results obtained in [redacted]6255 following the various treatment and immunization with VP16 and VP22 proteins-based nanoparticles.

I look forward to our collaborative work on this project.

Sincerely,

Christine E. McLaren

Christine E. McLaren, Ph.D.
Vice Chair and Professor, Department of Epidemiology
Director of Biostatistics

RESOURCE SHARING PLAN

Sharing Model Organisms: Research Resources generated with funds from this grant will be freely distributed upon request to qualified academic investigators for non-commercial research, to the extent that third-party patent rights and agreements permit and subject to availability.

Our unique HLA double transgenic mouse model of UVB-induced recurrent ocular herpes disease and any developed reagents will be made available to other NIH funded researchers *via* applicable University of California Irvine Material Transfer Agreements and/or licensing agreements through the UC Irvine Office of Technology Alliances.

Antigen Discovery Inc. will adhere to the NIH Grants Policy on Sharing of Unique Research Resources including the "Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIH Grants and Contracts", issued in December, 1999.

Specifically, material transfers to non-profit researchers would be made with no more restrictive terms than in the Simple Letter Agreements or the Uniform Biological Material Transfer Agreement (UBMTA) and without research through requirements to the extent permitted by any third-party patent or contract obligations. Should any intellectual property arise which Antigen Discovery Inc. decides to patent, we would ensure that the technology remains widely available to the non-profit research community in accordance with the NIH Principles and Guidelines.

The investigators have previously published their data in numerous publications and presented at worldwide scientific meetings, and it is their intention to continue to share data at the earliest opportunities throughout this research project. In particular: Results will be written up and sent for publication in relevant journals. The PI will seek to present publishable results at scientific conferences.

In accordance with NIH Data Sharing Policy, we will look to share data at the earliest opportunities throughout this research project, subject to intellectual property aspects.

Genome Wide Association Studies: Not applicable.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES